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IN HONOR OF

EDWARD ALLEN BOYDEN

IN CELEBRATION OF HIS 68TH BIRTHDAY

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In celebration of Edward Allen Boyden's 68th birthday, it is indeed a privilege for his colleagues and friends to express jointly their appreciation of his years of research and of his outstanding contributions to science including twenty years of service as editor of The Anatomical Record, 1928–48. Doctor Boyden, Professor and Head of the Department of Anatomy, University of Minnesota Medical School, is a man of broad interests, unsurpassed standards of excellence, and rare twofold competence as morphologist and experimentalist. In spite of his heavy load of teaching and administration, he continues to expand the frontiers of science through fundamental research.

His grandfather and his father were successively the third and fourth presidents of the Bridgewater (Mass.) State Teachers College (1860–1906 and 1906–1933) from which Allen was graduated in 1907. His early attraction to zoology is attributable in part to his father's interest in the natural sciences. Allen went to Harvard University where he was granted the bachelor's degree in 1909 and the master's in 1911.

A year at Freiburg, 1911–12, brought him under the influence of Keibel and of an anatomical institute, and shifted his major interest from zoology to medical anatomy. His experience abroad also accentuated his interest in the pictorial arts, in music and in the peoples of the earth. In London, on his way home, he purchased from an artist her copy of a Vermeer, and went without meals for the remaining

This welcome tribute to our affectionately esteemed predecessor is the result of a suggestion by a group of Doctor Boyden's close friends and associates, catalyzed by a small committee whose members modestly wish to remain anonymous. The photograph for the frontispiece was provided by Doctor Boyden's family: his wife, Mrs. Margaret Hilsinger Boyden, and two children, Arthur Clarke Boyden and Mary S. Boyden, M.D. Many of the papers appearing in this volume are invited contributions by Doctor Boyden's present and past associates. — EDITOR.

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days before boarding ship; his skill as a draftsman has always been an important part of his professional effectiveness. Mrs. Frederic T. Lewis recalls that at Freiburg he sang with a chorus some of the operas and that he first met his wife on the boat coming home. After marriage, their special pleasures included the playing of piano duets.

He has served in six American universities. At the Harvard Medical School from 1912 to 1926, where he received his Ph.D. (Med. Sc.) degree in 1916, he served as instructor in comparative anatomy for three years and assistant professor for seven. Two short leaves of absence were devoted to teaching and research at Stanford University and the University of California. Next came three years at the University of Illinois College of Medicine, as professor of anatomy, during which period he accepted the editorship of The Anatomical Record. Then he accepted an invitation to reorganize the Department of Anatomy at the University of Alabama, and remained there for two years. His move to the University of Minnesota in 1931 permitted the extension of an interest which was first aroused during the course of examining some 10,000 mammalian livers in the abattoirs around Boston; there he had encountered "kosher cutters," from the local synagogues, who informed him that anomalies such as he was seeking were already described in the Babylonian Talmud. At Minnesota, he assisted in translating that portion of the 16th-century codification of the Talmud which deals with anatomy. As a by-product of this purely technical interest in the history of medicine, he acquired an insight into the ancient cultures of Judaism. Similarly, as a by-product of travels incident to study in Europe and to service in six American universities, he has come to the conviction that the strength of America lies in the diversity of its parts.

He has been awarded the William Beaumont Prize, University of Illinois, 1928, and the Gold Medal of the Southern Minnesota Medical Association, 1937. He has served as president of the Minnesota Pathological Society, 1943; as first vice-president of the American Association of Anatomists,

1946–47; as a member of its executive committee, 1939–45; as a member of its committee on nomenclature since 1946; and as a member of the editorial board of Acta Anatomica since 1946.

He is currently working nights and weekends at his book on the lung, which he plans to finish before the summer of 1954. His hobbies include classical music, horseback riding with his daughter, morning runs with his Irish Setter, a boat on the Mississippi and two grandchildren, Wendy (9) and Tommy (3). His fondness for horseback riding has persisted, in spite of an accident in his youth in which he sustained a broken nose; and his eyes still brighten when he recalls that later, when a rodeo came to Boston, he rode a bucking bronco for more than a minute!

Much of his published original work is the outgrowth of observations recorded in his doctoral dissertation: "An anatomical study of the 13-mm chick; a contribution to the comparative embryology of birds and mammals" (1916, Harvard College Library). His important fundamental studies include those on gill filaments, cloaca and caudal intestine. Also, he discovered that in chick embryos and duck embryos the caudal end of the neural tube originates by a process of cavitation of the tail-bud mass (see his "A Laboratory Atlas . . . ," figs. 3–5). Of his original studies, the best known are those of the urogenital system, extrahepatic biliary tract and segmental anatomy of the lungs.

His first work on the urogenital system was published in 1924. He deprived chick embryos of the growing tips of the Wolffian ducts, and noted that the mesonephros begins to function as early as the fourth day of incubation. In 1932, he presented a sound embryological explanation of agenesis of the human kidney.

His studies of the extrahepatic biliary tract are traceable to the finding of a pancreatic bladder in one of 25 cats prepared for a class in comparative anatomy (1922). One of his 1953 papers ("Humoral vs. neural regulation . . .") sum-

marizes 30 years of work; and the early advances are best presented in brief by his own words:

". . . [In 1923,] this writer . . . was able to show that in cats the gallbladder could be completely emptied by a meal of egg yolk and cream, of which egg yolk was found to be the most effective component. The next year (1924), Graham and Cole . . . published a method of visualizing the human gallbladder that revolutionized the diagnosis of cholecystic disease and opened the way for direct experimentation with the human gallbladder. In the fall of the same year a group of workers at the Peter Bent Brigham Hospital in Boston — unaware of the earlier work on the cat — first observed changes in the gallbladder shadow 'after a meal.' The cholecystograms were made by Milliken and Whitaker [1925] on Whitaker's gallbladder. Subsequently, this work was expanded [1925], but previous to that the writer requested permission of the group to try the effect of egg yolk on his own gallbladder. This was done [1925] (p. 334) and Whitaker also published this cholecystogram [1926] confirming the observation that egg yolk is the most effective food . . .

". . . Boyden [1926] had shown that the gallbladder of a fasting animal could be activated by injecting into its leg vein 10 cc of arterial blood from a cat which was digesting egg yolk, and that marked contractions could be induced even by transferring blood from a fasting animal to a test cat, or by autotransfusion. Two years later, Ivy and Oldberg [1928] discovered that an extract of the duodenal mucosa (cholecystokinin) could evacuate the gallbladder of a dog when injected intravenously . . ."

Allen Boyden's studies of the lung, begun in February of 1945, are outgrowths of a surgical staff conference in which he was asked by Owen H. Wangensteen to discuss the anatomy of segmental pneumonectomy. Search of the literature having failed to yield any comprehensive figures of dissections of the external and interlobar surfaces of the lung, Doctor Boyden was obliged to make preliminary dissections. The first paper of his series, based upon these dissections, appeared in De-

cember of 1945. In a 1948 paper with J. Gordon Scannell, the opening sentence reads, "It is common experience that when a given field has been explored and a working terminology devised, the real problems begin to assert themselves."

In further celebration of Professor Boyden's birthday, colleagues and friends have submitted notes of appreciation. Since space permits only partial quotation here, these notes are to be bound and presented to the honoree as a "supplement."

Barry J. Anson: "To few investigators and teachers of our generation are so many former students as deeply beholden as hosts of medical graduates are to Edward Allen Boyden. This is tantamount to saying that his straightforward pedagogic methods, based upon selfdiscipline, superabundant information and practicality in sense of ultimate responsibility to the sick, have brought renown and popularity to his instructional sessions in laboratory, dispensary and clinical conference. This widely-recognized power was nurtured by a scholarly environment contributory to its growth and maturation, and was spurred to fruition by a zealous and dedicated enthusiasm akin to that which illumines scientific advance in every era.

"Allen Boyden fitted with facility and naturalness into the tradition of a Department where grounding stemmed from the achievements of Charles Sedgwick Minot and Frederic Thomas Lewis. During these early years, and consistently thereafter, there is evidenced in all of Professor Boyden's writings a respect for the venerable status of anatomy which, being employed to broaden his own treatment of a subject, imparts the concept of a continuum, never assumes the aspect of an element grafted upon the main theme of discourse. Those of us whose attention was held by the special excellence and temporal breadth of the lectures delivered by the late Professor Lewis, will appreciate from what source Dr. Boyden received encouragement.

"Allen Boyden, during his Harvard years, and in the course of all subsequent research, based all conclusions upon ob-

served facts, multiplied to impressive and incontrovertible numbers. Although regardful of the contentions of others and respectful of the knowledge stored in scientific literature, Dr. Boyden never bowed in convenient subservience to either.

"To the student in the first year of his graduate program (in which capacity my memory of Allen is most indelible), instruction was formidable only because the standards were high and the goals lofty. Yet, so earnest was his teaching, so clearly serviceable its intent, that the discipline was accepted rather than imposed. Never could the student question the aims and wishes of his mentor; never was the presentation oblique, the manner offhand, the commentary vapidly 'intellectual.' Punctuality, diligence and conscientiousness were still requisites in laboratory decorum, and failure to heed these exemplified *mores,* would have seemed effrontuous. All of this bore a mark of Old World schoolroom behavior, which, nowadays, alas, is a sadly abandoned code.

"These qualities were Dr. Boyden's guides in his own program of work; and even a casual reading of his publications would reveal that the phases of his life are unified and noncompetitive among themselves, his abundant energies productively enchannelled. For more than forty years he has served the cause of Medicine with unremitting zeal. So secure is his position, so valuable his contributions, that for the further embellishment of honors already received, Allen Boyden need only be granted continuing fullness of academic opportunity."

Charles H. Danforth: "Through the many years of our acquaintance Allen Boyden's sensitive responsiveness to diverse aspects of life has impressed me as one of his most distinctive characteristics. In our association on the Stanford campus and in the laboratory, during occasional evenings before an open fire, at many meetings of the Anatomists, I have become aware of his sensitiveness alike to the many-tinted fogs of a California sunset, to the philosophies of men in generations that have passed, to the aspirations of students approaching new worlds of endeavor, and above all to the

eternal pervasive mystery of life itself, whether encountered in a growing mesonephric duct, a functioning gall bladder, an evolving fetal lung.

"Dr. Boyden has brought illumination to every subject he has investigated, continuing year after year to accumulate the added knowledge and insight which have combined to make him a recognized authority in one field after another. In addition to his own studies and those which he has directly inspired, he has made a significant contribution through the 62 volumes of The Anatomical Record for which he served as editor. Few not on his board, and those only to a degree, could have been aware of the effort that went into shaping these volumes into the monument that they are. The critical reading of each paper, the pertinent questions, the helpful suggestions, the firm but sympathetic restraint on the over-enthusiastic, the immature and the ill-informed have borne real, if intangible, fruit for here no less than in the class room and the seminar, Dr. Boyden has been, as he continues to be, a potent influence in shaping the quality and the direction of American Anatomy."

Franklin S. Du Bois: "The Alabama days were indeed happy ones. They began almost twenty-five years ago and I look back on them as a great privilege, a privilege to be associated with one of the greatest anatomists of our times and one of the kindest men who ever lived. My admiration and affection for Allen are very great indeed."

Harold S. Diehl: "Over the many years that Dr. Allen Boyden has been a member of the staff of the University of Minnesota Medical School he has typified the finest type of faculty member. He has been a devoted and stimulating teacher of both under-graduate and graduate students. He has personally and continuously carried on important scientific investigations and has encouraged, guided and cooperated in similar work on the part of graduate students and other members of the medical faculty. He has in addition been a distinguished scholar in broad fields of learning and has made splendid contributions to the general program in the

administration of the Medical School. Any institution is fortunate to have on its faculty men with Dr. Boyden's competence and devotion."

Chester H. Heuser: "I am one of Allen's most ardent admirers. We were teaching fellows in Dr. Minot's laboratory.

"An amusing incident occurred the day we attended Dr. Cannon's lecture. When a pigeon deprived of its cerebellum was brought to him, he explained that the animal would be unable to execute coordinated movements and pitched the bird up in the air to demonstrate. However the pigeon made a bee-line for an open window and disappeared. Allen whispered in my ear 'Absence of the cerebellum causes instant flight.'

"I have watched with much pride Allen's rise to eminence. His great accomplishments already mark him as one of the best anatomists."

George M. Higgins: "Certainly no other investigator has with as great care or meticulous study advanced our knowledge of the extrahepatic biliary tract as Allen Boyden. His interest in this mechanism has not been transient, but has continued for more than a quarter century.

"Working always with intense zeal, dedicating himself to a truthful understanding of the biologic principles which operate, and yet with humility and sympathetic understanding, he has built for himself a personal and scientific reputation worthy of our highest esteem."

Thomas E. Hunt: "[At Alabama] Dr. Boyden had a profound effect on the development of the school and left behind influences which are still felt. He especially advocated anything which would encourage research.

"His presentation of gross anatomy to medical students from a developmental and functional point of view was both scholarly and stimulating.

"We are grateful to him for making our lives a richer experience and our search for scientific truth a more exciting adventure."

Leo G. Rigler: "The contributions of Dr. Edward Allen Boyden to the field of roentgen diagnosis are the most important of those of any living anatomist. Of fundamental significance were his studies of the time factor and the degree of contraction of the gallbladder in normals, under the influence of various physiological stimuli, and of various abnormal states. It is obvious that his work in the field of the anatomy and physiology of the biliary tract has been invaluable to the radiologist.

"Dr. Boyden's later work on the normal anatomy and anatomical variations of the tracheo-bronchial tree has been a revelation to radiologists. This research has produced the most important contributions to radiological anatomy and to an understanding of the roentgen findings in the lungs and bronchi since the earliest studies of William Snow Miller. His contributions have made the roentgen diagnosis of the biliary tract and of the respiratory tract far more accurate and have added immensely to the contributions of radiology in the diagnosis of disease."

Ralph F. Shaner: "My earliest recollection of Dr. Boyden is that of a teaching fellow, clad in a manila brown laboratory coat, who divided his time between . . . [teaching] and peering down C. S. Minot's old microscope at chick embryos. His meticulous care was a revelation to a beginner like myself. Combined with a restless curiosity it produced noteworthy results.

"Under the Minot regime everyone in the department had his 'chore.' Dr. Boyden's was the photographing of the embryos that went into the Minot Collection. It was good practice; for he was to be the Anatomist's chore-boy as editor of The Anatomical Record. He deserves the thanks of every anatomist the world over for his discharge of such a thankless task."

Richard L. Varco: "There is many an apt phrase . . . one could offer on behalf of a tribute to those numerous and significant contributions of Dr. Edward A. Boyden. These works exist because of an academic lifetime filled by a generous

allotment each day to productive investigative work. Of recent years, happily, these scientific efforts have been devoted to the development of our superior understanding of the precise anatomical configurations of the lungs, their segments, and blood supply. His sustained efforts . . . have earned widespread recognition for his work among those surgeons dealing with the problem of pulmonary resection. Today's widely accepted principle — when operating upon the lung conserve as much pulmonary tissue as possible — derives much of its practicalness from this application of a thorough knowledge of the common patterns and variables of the broncho-vascular structures."

Owen H. Wangensteen: "One cannot talk long with Allen Boyden without appreciating his deep sense of scholarship. It pervades every fiber of him.

"Over a period of approximately ten years, Dr. Boyden has come about once a quarter to talk to our Saturday morning Surgical Conference on the anatomy of some operative procedure. The preparation which he put into those presentations was truly extraordinary. [His preparation for his discussion of the lung in 1945 led him] to devote himself with such persistence and devotion to his recent studies, which have proved so helpful to surgeons.

"In my opinion, Allen Boyden is one of the great students of anatomy of his generation. He has left his impress upon this province of knowledge as well as upon this Medical School. Medical students, justifiably, have had great admiration and affection for him because of his unusual capacity as a teacher."

Cecil J. Watson: "Allen Boyden's classic studies of the functional anatomy of the gall bladder stand out amongst his many contributions to science because they have been of such importance in physiology and clinical medicine. Another study of this same type is his unusually painstaking delineation of the sphincter choledochus. It is safe to say that his more recent studies of the . . . [lung] bid fair to be classified in the same category. It is already clear that they will prove of considerable significance from the standpoint of the surgi-

cal treatment of various pulmonary diseases, especially bronchogenic carcinoma."

Finally, the stature of a man is indicated in part by his own words. To an appreciative freshman medical student who had just finished the course in gross anatomy, Doctor Boyden recently wrote from his desk at home the following reply:

"Your kind letter is one that I shall always treasure. In working with a class like yours one is ever conscious of its potentialities and one's own inadequacy. And yet, here and there, the miracle happens. A spark is struck and the tinder catches on fire. When I was your age such a spark was struck for me by F. T. Lewis. I could never repay him, but I could try to hand it on; and that is all that any of us can do. We all stand on the innumerable shoulders of those who have gone before. Thank you again for letting me know that I have not altogether failed."

A CHRONOLOGICAL LIST OF PROFESSOR BOYDEN'S PUBLICATIONS

(some 50 abstracts and book reviews omitted)

1913 (With H. W. Rand.) Inequality of the two eyes in regenerating planarians. Zool. Jahrb. Abt. f. Zool. u. Physiol., *34:* 69–80.

1917 The origin of man in the light of post-Darwinian discoveries. Proc. Harriett Newell Soc. Dent. Res., Harvard Univ., *2:* 12–15.

1918 Vestigial gill-filaments in chick embryos with a note on similar structures in reptiles. Am. J. Anat., *23:* 205–236.

1922 The development of the cloaca in birds, with special reference to the origin of the bursa of Fabricius, the formation of the urodaeal sinus, and the regular occurrence of a cloacal fenestra. *Ibid.* 30: 163–202.

—— A typical pancreatic bladder developed from an accessory pancreas. Anat. Rec., *23:* 195–203.

—— The early development of the cloaca in ostrich embryos, with special reference to the reduction of the caudal intestine. *Ibid. 24:* 211–222.

1923 The gall bladder in the cat — its development, its functional periodicity, and its anatomical variation as recorded in twenty-five hundred specimens. *Ibid. 24:* 388 (abstract).

1924 An experimental study of the development of the avian cloaca, with special reference to a mechanical factor in the growth of the allantois. J. Exp. Zool., *40:* 437–472.

1925 The effect of natural foods on the distention of the gall bladder, with a note on the change in pattern of the mucosa as it passes from distention to collapse. Anat. Rec., *30:* 333–364.

1925 The problem of the pancreatic bladder — a critical survey of six new cases, based on new histological and embryological observations. Am. J. Anat., *36:* 151–184.

1926 (With L. R. Whitaker.) Observations on the function of the gall bladder. Am. J. Physiol., *76:* 199–200 (abstract).

—— A study of the behavior of the human gall bladder in response to the ingestion of food; together with some observations on the mechanism of the expulsion of bile in experimental animals. Anat. Rec., *33:* 201–256.

—— (With R. B. Fleming.) The origin of the vertebral and external carotid arteries in birds. *Ibid. 33:* 183–200.

—— (With Cyrus H. Fiske.) Nitrogen metabolism in the chick embryo. An embryological and comparative study of aberrant biliary vesicles occurring in man and the domestic animals. Am. J. Anat., *38:* 177–232.

—— Behavior of the human gall bladder during fasting and in response to food. Proc. Soc. Exp. Biol. and Med., *24:* 157–162.

1927 Sex differences in the contraction rate of the human gall bladder. *Ibid. 24:* 353–358.

—— Experimental obstruction of the mesonephric ducts. *Ibid. 24:* 572–576.

—— (With C. L. Birch.) Conditions affecting the emptying-time of the human gall-bladder. *Ibid. 24:* 827–831.

—— Gall bladder versus sphincter papillae. *Ibid. 25:* 99–100.

1928 Concerning the prevalent denial of functions long attributed to the gall bladder. Surg., Gyn. and Obst., *46:* 30–41.

—— (With A. M. Saunders.) Duodenal drainage of the human gall bladder. Proc. Soc. Exp. Biol. and Med., *25:* 458–462.

—— (With Louis Parmacek.) Reflex inhibition of the human gall bladder. *Ibid. 25:* 462–464.

—— (With C. L. Birch.) Emptying of the human gall bladder after saline cathartics. *Ibid. 25:* 840–842.

—— Editorial [on the Third Editorial Board and the standards of The Anatomical Record]. Anat. Rec., *39:* a–b.

—— An analysis of the reaction of the human gall bladder to food. *Ibid. 40:* 147–192.

1929 (With C. L. Birch.) Emptying of the gall bladder in children. Proc. Soc. Exp. Biol. and Med., *26:* 312–313. Tonus changes in the gall bladder induced by faradic stimulation. *26:* 314. Reaction of gall-bladder to faradic stimulation of stomach. *26:* 466–467.

—— A note on the origin of pancreatic bladders. J. Anat., *63:* 363–366.

—— Concerning the regular occurrence of glairy cysts in the amnio-allantoic wall of chick embryos. Anat. Rec., *43:* 165–170.

—— (With Elmer Roberts and Leslie E. Card.) Double vents, associated with congenital absence of the left kidney and persistence of the embryonic genital papilla, in the domestic fowl. *Ibid. 43:* 155–164.

—— (With C. L. Birch.) Reflex control of the gall bladder, with special reference to inhibitory impulses originating in the gastrointestinal tract. Proc. Inst. Med. Chicago, *7:* 222–224.

—— An inquiry into the cause of congenital absence of the gall bladder. Proc. Soc. Exp. Biol. and Med., *27:* 86–87.

1929 New aspects of the physiology of the gall bladder. Bull. Jefferson Co. Med. Soc. (Illinois), 1 (No. 11): 5–10.

1930 (With C. L. Birch.) Reaction of gall bladder to stimulation of gastro-intestinal tract. I. Response to substances injected into the duodenum. Am. J. Physiol., *92:* 287–300. II. Response to faradic excitation of stomach, small intestine and cecum. *92:* 301–316.

—— (With Eleanor A. Hunt.) Is the cystic bile resorbed in toto? Proc. Soc. Exp. Biol. and Med., *27:* 645–647.

—— Reaction of gall bladder to stimulation of visceral nerves. *Ibid. 27:* 647–648.

1931 Correlation of uric acid production with growth of kidney tubules in chick embryos. *Ibid. 28:* 625–626.

—— (With Eleanor A. Hunt and A. Hobson Davis.) Initial changes in the tunics of the gall bladder induced by experimental ligation of the cystic duct. Anat. Rec., *49:* 295–308.

—— Description of a horseshoe kidney associated with left inferior vena cava and disc-shaped suprarenal glands, together with a note on the occurrence of horseshoe kidneys in human embryos. *Ibid. 51:* 187–212.

1932 Unilateral renal agenesis in a 10-mm human embryo. Proc. Soc. Exp. Biol. and Med., *29:* 411–412.

—— Joannes Baptista Biancus, Redivivus. Am. J. Roentg., *27:* 423–424.

—— Congenital absence of the kidney. An interpretation based on a 10-mm. human embryo exhibiting unilateral renal agenesis. Anat. Rec., *52:* 325–350.

—— Emptying of the gall bladder in monkeys. Proc. Soc. Exp. Biol. and Med., *29:* 1104–1105.

—— Editorial [on retrenchment due to the Depression and on the view that the quality of an original contribution is not measured by its length]. Anat. Rec., *54:* 135.

—— The problem of the double ductus choledochus. An interpretation of an accessory bile duct found attached to the pars superior of the duodenum. *Ibid. 55:* 71–94.

1933 Species differences in the reactions of the mammalian gall bladder. Am. J. Physiol., *105:* 11 (abstract).

—— Laboratory Atlas of the 13-mm. Pig Embryo. First printed ed., The Wistar Institute Press, Philadelphia.

1934 (With L. G. Rigler.) Localized pain accompanying faradic excitation of the stomach and duodenum. Proc. Soc. Exp. Biol. and Med., *31:* 655–656.

—— (With L. G. Rigler.) A cholecystographic and fluoroscopic study of the reaction of the human gall bladder to faradic stimulation of the stomach and duodenum. Anat. Rec., *59:* 427–447.

—— (With Alice H. Fuller.) Anatomy and physiology of the gall bladder in children. Am. J. Dis. Child., *48:* 565–579.

—— (With L. G. Rigler.) Localization of pain accompanying faradic excitation of stomach and duodenum in healthy individuals. J. Clin. Invest., *13:* 833–851.

1935 The "Phrygian Cap" in cholecystography. A congenital anomaly of the gall bladder. Am. J. Roentg., *33:* 589–602.

1936 (With Sam A. Grantham, Jr.) Evacuation of the gall bladder in old age. Surg., Gyn. and Obst., *62:* 34–42.

—— The pars intestinalis of the common bile duct, as viewed by the older anatomists (Vesalius, Glisson, Bianchi, Vater, Haller, Santorini, etc.). Anat. Rec., *66:* 217–232.

—— (With Maude M. Gerdes.) Retardation of the gall bladder in pregnancy. Proc. Soc. Exp. Biol. and Med., *35:* 393–394.

—— A Laboratory Atlas of the 13-mm. Pig Embryo (prefaced by younger stages of the chick embryo). Revised (3rd) ed., The Wistar Institute Press, Philadelphia, 93 pp.

1937 The sphincter of Oddi in man and certain representative mammals. Surgery, *1:* 25–37.

—— (With Theodore M. Berman.) Evacuation of the gall bladder in peptic ulcer patients. Radiology, *28:* 273–282.

—— (With Raymond A. Schwegler, Jr.) The development of the pars intestinalis of the common bile duct in the human fetus, with special reference to the origin of the ampulla of Vater and the sphincter of Oddi. I. The involution of the ampulla. Anat. Rec., *67:* 441–468. II. The early development of the musculus proprius. *68:* 17–42. III. The composition of the musculus proprius. *68:* 193–220.

—— The fifty-third annual session of the American Association of Anatomists, Toronto, Canada, March 25 to 27. Surgery, *1:* 820–822.

—— (With Wallace P. Ritchie.) Evacuation of the gall bladder in patients with carcinoma of the stomach. Proc. Soc. Exp. Biol. and Med., *36:* 815–816.

1938 (With Maude M. Gerdes.) The rate of emptying of the human gall bladder in pregnancy. Surg., Gyn. and Obst., *66:* 145–156.

—— The extrahepatic biliary tract as a functional unit. Surgery, *3:* 260–261.

—— New observations on the musculature of the choledocho-duodenal junction. Univ. Minn. Med. Sch. Digest., *2:* 29–30.

1939 The fifty-fourth meeting of the American Association of Anatomists, University of Pittsburgh, Pa., April 14–16. Surgery, *4:* 316–318.

1940 The choledocho- and pancreatico-duodenal junctions in the chimpanzee. Anat. Rec., 76 (Suppl.): 9 (abstract).

—— (With S. I. Levin.) The Kosher Code of the Orthodox Jew. Being a literal translation of that portion of the sixteenth-century codification of the Babylonian Talmud which describes such deficiencies as render animals unfit for food (Hilkot Terefot, Shulhan 'Aruk); to which is appended a discussion of Talmudic anatomy in the light of the science of its day and of the present time. Univ. Minn. Press, Minneapolis. 243 pp.

—— (With J. A. Layne.) Evacuation of the gall bladder in patients with pernicious anemia. Proc. Soc. Exp. Biol. and Med., *43:* 534–535.

—— (With B. L. Kreilkamp.) Variability of the composition of the sphincter of Oddi. A possible factor in the pathologic physiology of the biliary tract. Anat. Rec., *76:* 458–497.

—— A volumetric analysis of young human embryos of the 10- and 12-somite stage. Contrib. to Embryol., Carnegie Inst. Wash., *28:* 157–191.

1941 The sphincter of Oddi. (Editorial.) Surgery, *9:* 443–446.

—— Dr. Clarence M. Jackson retires. Minn. Alumni Weekly, *41:* 23.

1941 (With Frank E. Johnson.) Effect upon the biliary tract of sectioning the splanchnic nerves. Proc. Soc. Exp. Biol. and Med., *47:* 399–400.

—— Hypertrophy of the sphincter choledochus. Surgery, *10:* 567–571.

—— (With Robert A. Huseby.) Absence of the hepatic portion of the inferior vena cava with bilateral retention of the supracardinal system. Anat. Rec., *81:* 537–544.

1942 The choledocho-duodenal junction. Pages 441–444 in Maximow-Bloom, Textbook of Histology, W. B. Saunders Co., Philadelphia.

—— Directions for dissection. Revised ed. of Dept. Manual, 31 pp., Univ. of Minn., Minneapolis.

1943 (With John W. Schulze.) The blood supply and innervation of the choledochoduodenal junction in the cat. Anat. Rec., *86:* 15–39.

—— (With Frank E. Johnson.) The effect of sectioning various autonomic nerves upon the rate of emptying of the biliary tract in the cat. Surg., Gyn. and Obst., *76:* 395–410.

—— (With George S. Bergh and John A. Layne.) An analysis of the reaction of the human gall bladder and sphincter of Oddi to magnesium sulfate. Surgery, *13:* 723–733.

—— (With Charles van Buskirk.) Rate of emptying of biliary tract following section of vagi or of all extrinsic nerves. Proc. Soc. Exp. Biol. and Med., *53:* 174–175.

1944 (With L. G. Rigler.) Initial emptying time of stomach in primigravidae as related to evacuation of the biliary tract. *Ibid. 56:* 200–201.

—— Congenital variations of the extrahepatic biliary tract. Minn. Med., *27:* 932–933.

—— (With John A. Layne.) Evacuation of gall bladder in female patients with pernicious anemia. Proc. Soc. Exp. Biol. and Med., *57:* 315–316.

—— (With John A. Layne.) The gall bladder in patients with pernicious anemia: a study of non-visualization and rate of emptying. Gastroenterol., *4:* 121–134.

1945 Editorial [on nomenclature]. Anat. Rec., *92:* 101–103.

—— The intrahilar and related segmental anatomy of the lung. Surgery, *18:* 706–731.

1946 (With J. Francis Hartmann.) An analysis of variations in the left upper lobes of fifty lungs. Am. J. Anat., *79:* 321–360.

—— The anatomical hazards of lingulectomy. Surgery, *20:* 828–829.

1947 Clarence Martin Jackson. 1875–1947. Anat. Rec., *98:* 315–324.

1948 (With J. Gordon Scannell.) An analysis of variations in the bronchovascular pattern of the right upper lobe of fifty lungs. Am. J. Anat., *82:* 27–74.

—— (With J. Gordon Scannell.) A study of variations of the bronchopulmonary segments of the right upper lobe. J. Thor. Surg., *17:* 232–237.

—— Introduction to Detailed Atlas of Head and Neck, by Raymond C. Truex and Carl E. Kellner. Oxford Univ. Press, New York, IX–X.

1949 (With Franklin R. Smith.) An analysis of variations of the segmental bronchi of the right lower lobe of fifty injected lungs. J. Thor. Surg., *18:* 195–215.

1949 (With Roger M. Berg and Franklin R. Smith.) An analysis of the segmental bronchi of the left lower lobe of fifty dissected, and ten injected lungs. *Ibid. 18:* 216–236.

—— A synthesis of the prevailing patterns of the bronchopulmonary segments in the light of their variations. Dis. Chest, *15:* 657–668.

—— Cleft upper lobes and split anterior bronchus. Surgery, *26:* 167–180.

—— Harold Lorraine Weatherford. 1891–1948. Anat. Rec., *105:* 124–125.

1951 (With C. J. Hamre.) An analysis of variations in the bronchovascular patterns of the middle lobe in fifty dissected and twenty injected lungs. J. Thor. Surg., *21:* 172–188.

—— (With Ronald M. Ferry.) Variations in the bronchovascular patterns of the right lower lobe of fifty lungs. *Ibid. 22:* 188–201.

1952 Frederic T. Lewis. 1875–1951. Anat. Rec., *112:* 137–152.

—— (With Louis B. Thomas.) Agenesis of the right lung. Surgery, *31:* 429–435.

—— The distribution of bronchi in gross anomalies of the right upper lobe, particularly lobes subdivided by the azygos vein and those containing pre-eparterial bronchi. Radiology, *58:* 797–807.

—— (With Frank E. Johnson.) The effect of double vagotomy on the motor activity of the human gall bladder. Surgery, *32:* 591–601.

1953 A critique of the international nomenclature on bronchopulmonary segments. Dis. Chest, *23:* 266–269.

—— Humoral vs. neural regulation of the extra-hepatic biliary tract. Minn. Med., *36:* 720–723.

—— Observations on the anatomy and development of the lungs. Journal Lancet, *73:* 509–512.

—— (With Martha Pitel.) Variations in the bronchovascular patterns of the left lower lobe of fifty lungs. J. Thor. Surg., *26:* 633–653.

—— Lateral views of the segmental bronchi and related pulmonary vessels in an injected preparation of the lungs. Radiology, *61:* 183–188.

In press: (With L. J. Wells.) The early development of the bronchopulmonary segments. Anat. Rec., 118 (Suppl.). (Abstract.)

—— Segmental Anatomy of the Lung. P. Blakiston's Son and Co., Philadelphia. (This monograph, to appear in 1954, will have 124 figures, 72 of which are in black and white and 64 in color.)

ELECTRON MICROSCOPY OF MOTOR NERVE CELLS FOLLOWING SECTION OF AXONES[1]

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FOUR FIGURES

INTRODUCTION

In previous studies with the light microscope (Hartmann, '48, '49), the basic fuchsin technique of Fain and Wolfe ('44) was used to demonstrate mitochondrial changes following section of axones. It was found that motor cells of the sciatic and hypoglossal nerves showed an increase in fuchsinophilic granulation on the operated side after unilateral neurotomy. The increase was maximal on the 11th postoperative day, when the number of stainable granules, presumably mitochondria, on the homolateral side was nearly double that of the control side. Because the average diameter of the mitochondria (3000 Å) is so close to the resolving limit of the light microscope, by the use of staining reactions alone it was impossible to be certain that all red-staining granules were in fact mitochondria. Other possibilities, however unlikely, could not be positively ruled out. Thus, the only argument against the view that some of the increased granulation might be due to particles of degraded Nissl substance responding to the mitochondrial stain was the fact that the granules were found to be as numerous in the Nissl-free axone hillock as elsewhere in the cell. Some other criterion for positive identification of mitochondria was therefore necessary.

[1] This investigation was supported by a research grant (MH-388) from the National Institute of Mental Health of the National Institutes of Health, Public Health Service, and by the Graduate Medical and Cancer Research Fund of the University of Minnesota.

During the course of these experiments, a second problem presented itself. It concerned the role played by the nuclear membrane in nucleotide metabolism, since the ultraviolet microspectrographic studies of Hydén ('43) had related much of the protein synthesis in nerve cells to the nucleolus. In this instance also, the resolving power of the light microscope, even in the ultraviolet part of the spectrum, was insufficient for adequate analysis of this problem.

At this juncture, it became apparent from the work of Pease and Baker ('48), Baker and Pease ('49) and of Newman, Borysko and Swerdlow ('49a, '49b) that sections sufficiently thin for electron microscopy could be consistently prepared. Following this work, descriptions of cellular ultrastructure, including details of mitochondrial organization, began to appear in the literature. Bang and co-workers ('52) established the validity of identifying mitochondria in electron micrographs of osmium-fixed tissue by comparing phase contrast micrographs of living cells with electron micrographs of the same cells after fixation.

The way seemed clear for a simultaneous attack on problems concerning the mitochondria and the nuclear membrane by repeating the earlier axone section experiments, but using the electron microscope to analyze the results. As a necessary preliminary, the range of ultrastructural variation in the central nervous system of normal rats was explored (Hartmann, '53). The present account, therefore, deals with changes in the mitochondria and in the nuclear membrane in motor nerve cells following section of axones, as revealed at the level of electron optical resolution. It is possible to conclude that the increased fuchsinophilic granulation seen in the writer's previous experiments was in fact due to increased numbers of mitochondria in cells affected by the operation, since comparable results have been obtained with the electron microscope, and since mitochondria are positively identifiable in electron micrographs on the basis of size, shape, electron density and internal structure. The ultrastructural alterations that have been observed in the nucleus give some support to

the view that the nuclear membrane in nerve cells may be not so much a passive barrier as a chemically active zone or interface (Hartmann, '52a, '52b, '53).

MATERIALS AND METHODS

A series of 35 young adult male rats was subjected to unilateral hypoglossal neurotomy under ether anesthesia. Cells of the hypoglossal nucleus were studied in thin sections in the electron microscope through the 15th postoperative day, with particular reference to the mitochondria and nuclear membrane. The methods of fixation and embedding were those already described in detail (Hartmann, '53). All sections were cut with the glass knife (Latta and Hartmann, '50),[2] at thicknesses ranging between 0.05 and 0.1 μ. All studies were made with the RCA model EMU electron microscope, equipped with self-bias gun, intermediate lens, and standard objective aperture.

OBSERVATIONS

Mitochondria

The electron microscope study fully confirms the earlier observations made with the light microscope, inasmuch as the number of identifiable mitochondria is increased on the operated side as contrasted with the control side (figs. 1 and 2). No attempt was made to count the mitochondria, because of the extreme difficulty of obtaining controlled serial sections within the requisite range of thickness, and because inspection of the micrographs gave no reason to doubt the validity of the numerical data obtained with the light microscope (Hartmann, '48).

The mitochondria seen in increased numbers on the operated side are structurally identical with those of the control side. Reference has already been made to a certain degree of structural variation among mitochondria in normal cells (Hartmann, '53), and the chondriosomes seen on the operated

[2] Knives of selected glass were obtained from the Brin Glass Co., Minneapolis.

side exhibit a similar spectrum, except that a greater proportion of them are elongated in form and less dense to the electron beam, apparently because their constituent granules are less closely packed (fig. 2). It seems likely that these mitochondria are recently formed. If this is true, the fact that so many of them are filamentous is of interest in relation to their possible mode of formation, i.e., it appears unlikely that they arise by fragmentation of pre-existing mitochondria. In fact, in cells on the control side, filamentous forms are in the minority as contrasted with spherical forms. As is the case in normal nerve cells, only a small proportion of mitochondria from either the operated or control side show transverse striations resembling those described by Palade ('53), Sjöstrand ('53) and Sjöstrand and Rhodin ('53), as being characteristic of mitochondria in various other types of cells. The insert in figure 2 shows a group of mitochondria, one of which has a transversely striated appearance. The fact that filamentous forms frequently appear ragged at one end or both, is probably due to at least two factors. One is that elision of marginal irregularities whose dimensions are too close to the resolving limit (von Borries and Kausche, '40; Seeliger, '48) makes all mitochondria look "smooth" in the light microscope. The other is that long or wavy mitochondria cannot be expected to coincide with the plane of even 0.1 μ sections throughout their length, and where they are truncated as they pass out of the plane of section, the electron microscope resolves such irregularities as may be present.

It should be stated that the disparity in mitochondrial content between experimental and control cells is not absolute. In a sufficiently large series of micrographs, some control cells will be found that appear to have as many mitochondria as some experimental cells, and conversely. However, when over 200 micrographs were sorted as representing cells from one side of the brain stem or the other, purely on the basis of mitochondrial appearance, a subsequent check on the actual origin of the micrographs showed the sorting to

have been nearly 95% accurate. The 5% or so of control cells that look like experimental ones could have been metabolically stimulated by unknown factors not intended in the experiment; conversely, the few experimental cells that look normal may not have been in a state of maximal mitochondrial concentration at the time of fixation.

Nuclear membrane and nucleoplasm

A characteristic alteration in the nuclear membrane in cells on the operated side was noted, the severity of which was not correlated with the length of time elapsing after section of the hypoglossal nerve. The change is that from a thin, electron-dense line or pair of lines at the nucleo-cytoplasmic interface (fig. 3) to a broad band or zone of granules (fig. 4). These granules are indistinguishable, on the basis of size and electron density, from those making up most of the mitochondria. Many cells from animals killed 1 to 5 days after operation show an apparent depletion of nucleoplasm along with the granular transformation of the nuclear membrane, but this is not a universal finding. The electron density of both nucleus and cytoplasm varies so disproportionately with small variations in section thickness that until better means of controlling and measuring this parameter are at hand, it is impossible to be sure, except in extreme cases, whether the depletion of nuclear material is real or only apparent. In the same way, a reliable assessment of nucleolar diameter would be interesting, but is next to impossible to achieve in such thin sections, since so many of them could be cutting the nucleolus eccentrically. Thus, the ultraviolet microspectrographic findings of Hydén ('43) can be only partially verified, due to technical conditions. Nevertheless, the granular appearance of the nuclear membrane in the experimental cells is of interest from the viewpoint of possible nucleocytoplasmic exchange. Caspersson ('50), on the basis of studies with ultraviolet absorption, states that in normal cells, proteins diffuse from the nucleolus toward the nuclear mem-

brane, and that on the outside of the latter an intensive production of ribose nucleotides takes place. As Hydén ('50) points out, in the regenerating neuron the cell body must produce about 1000 times its own volume of cell substance. Both authors suggest that the nucleolus is a focal point of protein synthesis. The fine structure of the nuclear membrane is not amenable to analysis with the ultraviolet microscope, however, and such studies can with profit be supplemented by electron optical investigations of experimentally altered nerve cells.

No evidence for a system of regularly arranged pores in the nuclear membrane, even in the experimental cells of the present study, was found. Indentations similar to those seen in nuclear membranes of normal nerve cells appear with about the same frequency in the experimental cells, but in the latter are usually deeper (cf. figs. 3 and 4).

DISCUSSION

In view of the rapidly accumulating evidence on the biochemical significance of mitochondria (Schneider, '53), the positive identification of these cytoplasmic inclusions in electron micrographs, in confirmation of the earlier light microscope studies, is of self-evident importance. It seems reasonable to suppose that the neurons containing increased numbers of mitochondria are in a regenerative phase, but from the present experiments it is not possible to conclude what type of metabolic activity, e.g., respiration, is principally involved. It may be, as Bourne ('51) suggests, that increased numbers of mitochondria in nerve cells following section of axones are associated with the regeneration of myelin, but other experiments will be necessary to test this hypothesis. At any rate, the appearance of the experimental cells in the present study suggests hyperactivity instead of degeneration. It would doubtless be a profitable line of inquiry to follow the course of these observed ultrastructural changes well beyond the 15th postoperative day.

The reason why so few mitochondria in either the experimental or control cells in this experiment show internal structure similar to that described by Palade ('53), Sjöstrand ('53) and by Sjöstrand and Rhodin ('53) is not immediately clear. As predicted by Schmitt ('49), the problem of fixation is even more vexing to electron microscopists than it was for so long to workers in light microscopy. The preparative techniques that have been evolved to secure sections thin enough for good resolution with an electron optical system have without doubt introduced a new set of artefacts that have as yet been only imperfectly evaluated. Thus, the differences between the mitochondria seen in nerve cells by the writer and those seen in other cell types by others may be related to the cytology of the nerve cell which is unique in several other respects, or the differences may be due to factors of fixation, embedding or even sectioning that are not yet understood. Objective criteria for evaluating the quality of fixation at the ultrastructural level are still lacking, but there is room to hope that the electron microscope itself may prove to be the means of supplying them (Schmitt, '49). However, the present work can legitimately prescind from the more fundamental problem regarding the true structure of mitochondria in the living cell. The chief concern here is whether or not changes in the mitochondrial content of motor nerve cells can be observed following section of their axones. Since both sides of the central nervous system of these experimental animals were subjected (as far as can now be known) to identical preparative treatment, the observed increase in mitochondria on the operated side is more probably to be ascribed to axone section than to conditions of technique.

The evidence from the present work concerning the possible origin of new mitochondria is not clear-cut. Miller ('53) has adduced strong evidence that in the adrenal cortex, elongated mitochondria may segment to increase the number of spherical forms. As already mentioned, most of the mitochondria interpreted as being newly formed in the present work are of the filamentous type, and no evidence of fragmentation

was observed at the level of resolution attained. Miller further states that our knowledge is still incomplete as to whether mitochondria may subserve different functions in different cells, and we may here be dealing with a case in point. Schneider ('53) has reviewed the evidence pointing toward biochemical heterogeneity of mitochondria, a phenomenon which could conceivably be reflected by structural heterogeneity as well.

No stages clearly indicative of mitochondria in the process of formation appear in the electron micrographs. Possibly a series with intervals of less than 24 hours after operation would show such stages. The possibility that the filamentous mitochondria might arise from the submicroscopic cytoplasmic fibrils suggested itself, but is discounted by the fact that many cells were seen containing large numbers of mitochondria but with the cytoplasmic fibrils still in evidence. The evidence available in this study suggests that the mitochondria arise by accretion of submicroscopic cytoplasmic particles at or near the nucleo-cytoplasmic interface. The granules of the thickened nuclear membrane, of the same size and electron density as the constituent granules of the mitochondria, provide an interesting basis for speculation in the light of Bensley's ('47) suggestion that chondriosomes may be temporary aggregates. Such a view is not irreconcilable with the ultrastructural pattern revealed in mitochondria by the electron microscope, i.e., the fact that a complex internal structure may be demonstrable in them is not by itself sufficient reason for concluding that the chondriosomes must be permanent cellular organelles. The well-known phenomena of cellular differentiation, dedifferentiation and redifferentiation may each be characterized by complex but transitory cellular architecture. The secretory cycles of the anterior hypophysis are an example of temporary structural entities that may prove quite as highly organized at the submicroscopic level as are the mitochondria. There is no evidence in the present experiments that is inconsistent with Bensley's hypothesis, or even with the view that the constituent particles

of the mitochondria might have migrated from the nucleoplasm.

Such considerations as those above lead naturally to the problem of the nuclear membrane. The similarity between the submicroscopic granules of the nucleoplasm and those of the thickened nuclear membrane in experimental cells is interesting in the light of the hypothesis (Hartmann, '52a, '53) that the nuclear membrane plays an active rather than a passive role in the economy of the nerve cell. If at least some of the observed diminution in nuclear density in these experiments is real, it seems possible that in metabolically stimulated nerve cells, certain compounds synthesized in relation to the nucleolus may undergo transformation as they migrate to the cytoplasm. In such a case, the nuclear membrane could represent, not a physical barrier, but a relatively static phase in the transformation of such migrating materials. The structural variability among nuclear membranes of nerve cells from normal animals (Hartmann, '53) could be explained as characterizing cells in which protein synthesis and transport were proceeding at varying rates, or which were in various stages of the process at the time of fixation. The total absence of regularly arranged pores in the nucelar membranes of experimental cells seems to rule out the hypothesis of direct nucleo-cytoplasmic outflow as a characteristic phenomenon of adult nerve cells.

SUMMARY

1. In light microscope observations, previously reported by the writer, an increase in fuchsinophilic granules in motor nerve cells following section of axones was described. These granules were found to be structurally and tinctorially similar to mitochondria in normal nerve cells, but the resolving power of the light microscope did not permit unqualified identification of all new granules as mitochondria.

2. In the present study, a series of 35 rats was subjected to unilateral hypoglossal neurotomy, and cells of the hypoglossal nucleus were studied in sections in the electron mi-

croscope through the 15th postoperative day, with particular reference to mitochondria and the nuclear membrane.

3. The earlier observations made with the light microscope were fully confirmed, inasmuch as mitochondria are identifiable in the electron microscope on the basis of size, shape, electron density and internal structure.

4. Although no clear evidence regarding the origin of new mitochondria was found, there is some reason to think that they may arise in nerve cells by accretion of submicroscopic granules at or near the nucleo-cytoplasmic interface, rather than from the cytoplasmic fibrils or by fragmentation of preexisting chondriosomes.

5. The single or double thin, dense line representing the nuclear membrane in sections of normal cells is replaced by a relatively wide band or zone made up of submicroscopic granules.

6. No evidence for a system of regularly arranged pores in the nuclear membrane was found in either control or experimental cells.

7. It is suggested that in metabolically stimulated nerve cells, the nucleoplasm may undergo transformation while migrating to the cytoplasm, with the thickened, granular nuclear membrane representing a relatively static phase of the transformation.

LITERATURE CITED

BAKER, R. F., AND D. C. PEASE 1949 Improved sectioning technique for electron microscopy. J. Appl. Phys., *20:* 480.

BANG, F. B., G. O. GEY, K. PAZAMENAS AND P. SAPRANAUSKAS 1952 The validity of cellular structure as seen with the electron microscope. J. Appl. Phys., *23:* 162.

BENSLEY, R. R. 1947 On the nature of the pigment of mitochondria and of submicroscopic particles in the hepatic cell of guinea pig. Anat. Rec., *98:* 609–619.

VON BORRIES, B., AND G. A. KAUSCHE 1940 Übermikroskopische Bestimmung der Form und Grössenverteilung von Goldkolloiden. Koll. Z., *90:* 132–141.

BOURNE, G. H. 1951 Cytology and Cell Physiology. Ed. by G. H. Bourne. Academic Press, Inc., New York, Chap. VI, 232–286.

CASPERSSON, T. O. 1950 Cell Growth and Cell Function. W. W. Norton and Co., Inc., New York.

FAIN, W. R., AND J. M. WOLFE 1944 A cytological stain for the anterior pituitary gland involving the use of basic fuchsin. Anat. Rec., *90:* 311–314.

HARTMANN, J. F. 1948 Mitochondria in nerve cell bodies following section of axones. Anat. Rec., *100:* 49–59.

———— 1949 Mitochondria in cell bodies of the hypoglossal nucleus and of spinal ganglia following section of nerves. Anat. Rec., *103:* 541–542.

———— 1952a Structure of nuclear membrane in sections of central nervous system. J. Appl. Phys., *23:* 163–164.

———— 1952b Electron microscopy of nuclei in nerve cells. Anat. Rec., *112:* 340.

———— 1953 An electron optical study of sections of central nervous system. J. Comp. Neur., *99:* 201–249.

HYDÉN, H. 1943 Protein metabolism in the nerve cell during growth and function. Acta Physiol. Skand., *6,* Suppl. 17: 1–136.

———— 1950 Spectroscopic studies on nerve cells in development, growth and function. In Genetic Neurology, ed. by P. Weiss. The University of Chicago Press, Chicago, pp. 177–193.

LATTA, H., AND J. F. HARTMANN 1950 Use of a glass edge in thin sectioning for electron microscopy. Proc. Soc. Exp. Biol. and Med., *74:* 436–439.

MILLER, R. A. 1943 The relation of mitochondria to secretory activity in the fascicular zone of the rat's adrenal. Am. J. Anat., *92:* 329–360.

NEWMAN, S. B., E. BORYSKO AND M. SWERDLOW 1949a New sectioning techniques for light and electron microscopy. Science, *110:* 66–68.

———— 1949b Ultra-microtomy by a new method. J. Res. Nat. Bur. Stand., *43:* 183–199.

PALADE, G. E. 1953 The fine structure of mitochondria. An electron microscope study. J. Histochem. and Cytochem., *1:* 188–211.

PEASE, D. C., AND R. F. BAKER 1948 Sectioning techniques for electron microscopy using a conventional microtome. Proc. Soc. Exp. Biol. and Med., *67:* 470–474.

SCHMITT, F. O. 1949 Some commentaries on electron microscopy as applied in biology. Fed. Proc., *8:* 530–535.

SCHNEIDER, W. C. 1953 The biochemical constitution of isolated mitochondria. J. Histochem. and Cytochem., *1:* 212–233.

SEELIGER, R. 1948 Punctauflösung und polygonal begrenzte Objekte im Elektronenmikroskop. Optik, *3:* 315–319.

SJÖSTRAND, F. S. 1953 Electron microscopy of mitochondria and cytoplasmic double membranes. Nature, *171:* 30–32.

SJÖSTRAND, F. S., AND J. RHODIN 1953 The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy. Exp. Cell Res., *4:* 426–456.

PLATE 1

EXPLANATION OF FIGURES

Scale indicates 1 μ.

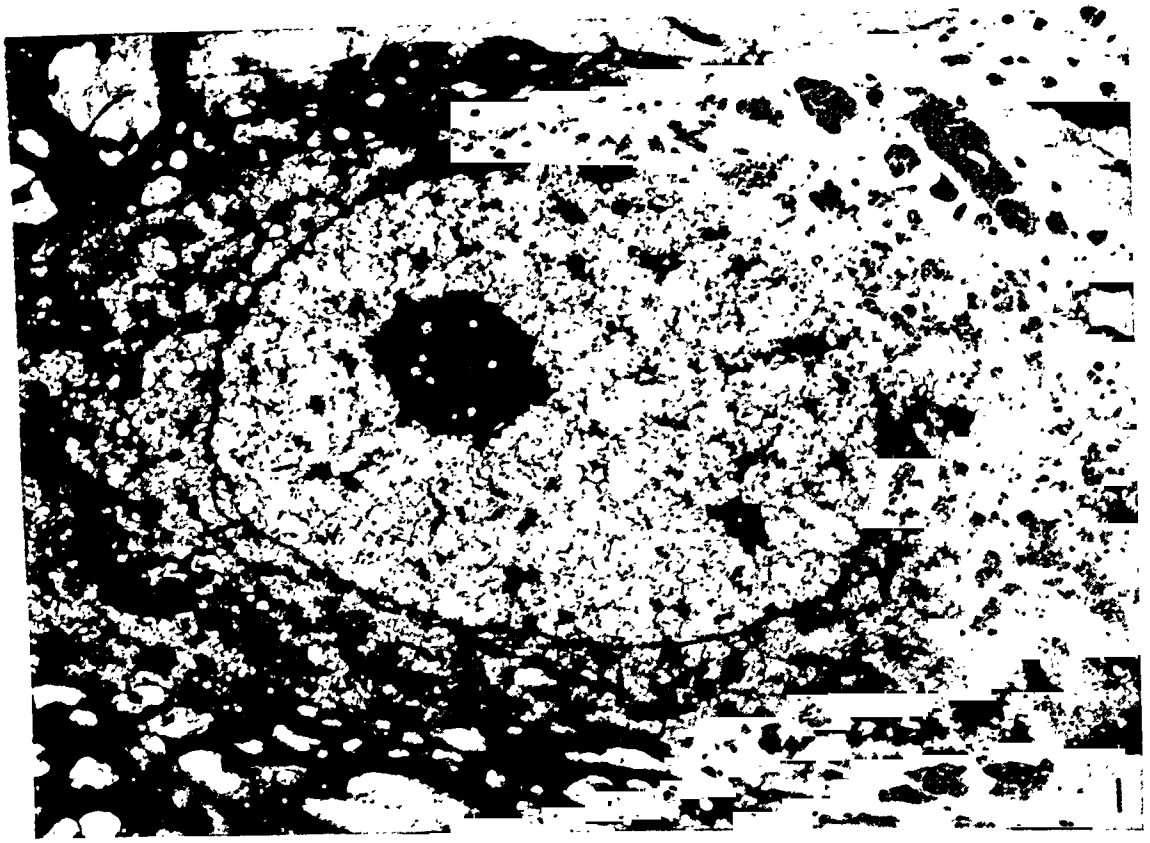
1 Cell from hypoglossal nucleus on control side of rat 10 days following neurotomy. A few dense mitochondria appear in the cytoplasm. × 6400.

2 Cell from operated side 7 days after neurotomy. A few dense mitochondria and numerous less opaque forms are present in the cytoplasm. The granular transformation of the nuclear membrane can be seen. × 16,500.

Insert: A group of mitochondria in the cytoplasm of a cell on the operated side, 8 days after operation. One of them shows transverse striations. × 18,550.

ULTRASTRUCTURE FOLLOWING NEUROTOMY

J. FRANCIS HARTMANN



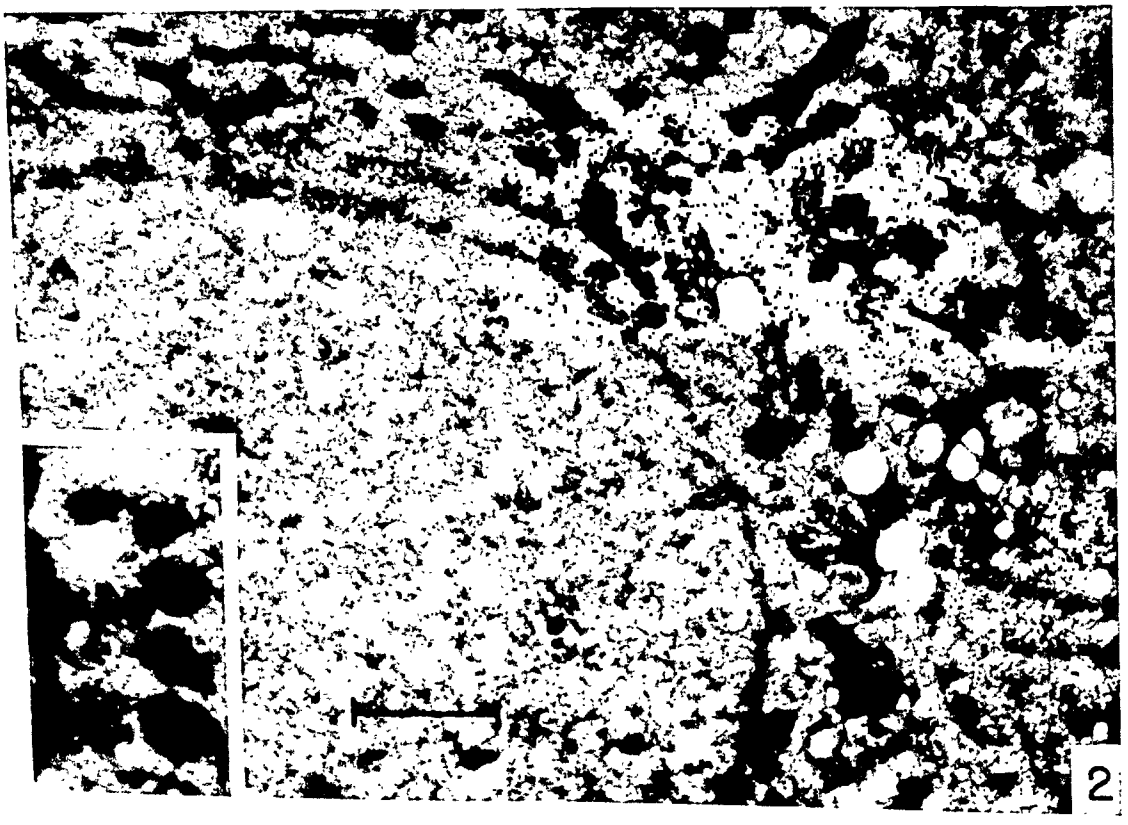


PLATE 2

EXPLANATION OF FIGURES

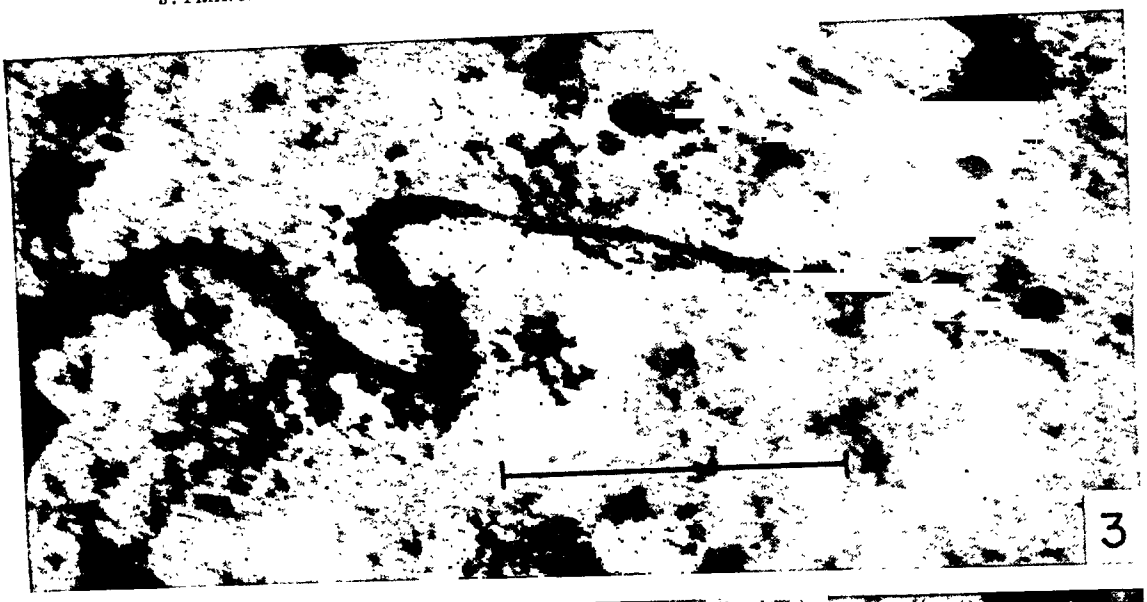
Scale indicates 1 μ.

3 Cell from hypoglossal nucleus of control side, 7 days after operation. The nuclear membrane is represented by a pair of electron-dense lines, and is very thin. The indentation is similar to those frequently found in normal nerve cells throughout the central nervous system. × 37,550.

4 Portion of nuclear membrane in cell of hypoglossal nucleus on operated side, 5 days after neurotomy. Even at this magnification, lower than that of figure 3, the thickened, granular appearance of the nuclear membrane is evident. × 23,350.

ULTRASTRUCTURE FOLLOWING NEUROTOMY

J. FRANCIS HARTMANN



TISSUE MAST CELLS IN HUMAN UMBILICAL CORD, AND THE ANTICOAGULANT ACTIVITY OF DRIED EXTRACTS OF CORDS AND PLACENTAE

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NINE FIGURES

INTRODUCTION

Tissue mast cells are an expected cellular constituent of most types of connective tissue, but they often are not recognized even when they are particularly numerous because the various routine water-soluble fixatives allow dissolution of the water-soluble metachromatic granules. Because of the mucous nature of Wharton's jelly, the latter might be expected to contain numerous mast cells. The present investigation was directed towards demonstrating the presence of mast cells in the umbilical cord of humans. The findings led to preliminary investigations of the anticoagulant activity of dried water extracts of the umbilical cord and placenta.

MATERIALS, METHODS AND OBSERVATIONS

Our original interest in this problem was purely histological. Although Lehner described tissue mast cells in the umbilical cord in '24, their presence in this location is not acknowledged in textbooks of histology (Maximow and Bloom, '52). In the present investigation, first efforts were directed toward identification of tissue mast cells in dry smear preparations of the mucous connective tissue of the umbilical cord. Fresh human

umbilical cords [1] were obtained; the amnion was slit with a blade; and smears were made from the exuding mucus. The smears were dried rapidly and stained with Wright's stain and/or with toluidine blue. Grossly, smears of this type had a rich reddish purple color, the entire semi-amorphous ground substance being metachromatic. Scattered in this substance numerous tissue mast cells were usually found. In addition, the "fibroblasts" of mucous connective tissue with their varying bizarre shapes were easily identified. A large percentage of these cells contained metachromatic granules which varied in number, size, shape and state of preservation. Many of the "fibroblasts" contained annular granules with colorless central areas and also showed vacuolization of the cytoplasm; both of these phenomena are known to occur in blood or tissue mast cells when the granules have undergone partial dissolution. Twenty-two umbilical cords have been studied. Smears of all of these cords have shown tissue mast cells.

EXPLANATION OF FIGURES

Figures 1 through 5 show various types of cells which contain deeply metachromatic granules as they appear in dry smears of Wharton's jelly stained with toluidine blue or Wright's stain. Figure 1 shows a relatively low-power view of three tissue mast cells. Often, in dry smears, 4 or 5 of these cells would be found in a single low-power field. The scratches in the background (fig. 1) give an indication of the stainability of the ground substance. Figure 2 shows a typical tissue mast cell comparable to the three cells in figure 1. These cells are comparable to the tissue mast cells found in imprints of human bone marrow, lymph nodes, and spleen. Figure 3 shows a cell with deeply metachromatic granules which vary remarkably in size. Some are as small as those

[1] Cords were obtained from both primiparous and multiparous mothers. In some instances it was possible to accomplish techniques necessary to the present problem as soon as 5 or 10 minutes after delivery of the placenta. However, results were positive even when a longer time elapsed (one-half to two hours) following delivery.

seen in the mast cell in figure 2; others are as large as those seen in the cells shown in figure 4. The variability in the size and degree of metachromasia of mast cell granules was described and illustrated by Downey ('13). Downey's colored figures 21 through 23 show mast cells in section preparations from the mucous membrane of the turbinate bone; the variation in size and color of the granules is clearly apparent. Zollinger ('50a, '50b) has described and illustrated the variability in size, contour and metachromasia of mast cell granules. With phase and with ordinary microscopy, he has observed and photographed the dissolution of mast cell granules under the influence of distilled water or after prolonged exposure to physiological saline.

Figures 4 and 5 show cells with relatively abundant cytoplasm. These cells have irregular cell outlines, granules which vary in size and shape, and varying numbers of vacuoles as well as evidence of partial dissolution of granules. Cells with similarly abundant cytoplasm containing vacuoles and metachromatic granules of varying sizes are also visible in sections. In the latter preparations, some of the cells could be considered to be fibroblasts, clasmatocytes or histiocytes as they showed stellate, ameboid or irregularly angular cell bodies. It seems possible that some of the "stellate-fibroblasts" (Maximow and Bloom, '52) or some of the other connective tissue cells of Wharton's jelly which can be shown to contain metacromatic granules with appropriate fixation and staining are potential mast cells.

Figures 6 through 9 show tissue mast cells in sections. Umbilical cords were fixed in 8% basic lead acetate, sectioned at 6 μ, and stained with Dominici's stain (eosin, orange G and toluidine blue) or with toluidine blue. Mast cells could be found throughout the mucous connective tissue. The darkest small black spots in figure 6 are mast cells. Some of the more lightly stained cells are comparable to the one shown in figure 7. This cell contains both vacuoles and metachromatic granules; this is only partially evident in the photomicrograph, the cells being particularly difficut to photograph

because of their thickness. Figure 8 shows the metachromatic granules in one side of the cell and super-imposed upon the nucleus. Figure 9 is the clearest of the photomicrographs obtained; here even the variation of size of the granules can be seen.

Extraction and clotting studies

Because the number of mast cells in Wharton's jelly seemed so great, attempts to extract the substance they presumably contain were made. In the initial experiments blood was milked from the umbilical vein; in the later experiments blood was withdrawn from the vein by means of a large needle and syringe until both the cord and the placenta were as free of blood as possible. The cord was severed from the placenta and cut into segments about one inch in length. The amnion of the cord was slit in numerous places in order that Wharton's jelly be freely exposed to the extracting fluid. Eight of the cords were allowed to remain in 100 to 300 cm^3 of distilled water at room temperature for 10 to 12 hours. Six of the cords were extracted with 0.9% NaCl (5 cm^3, g. tissue) as well as with water. The three earliest cords studied were not extracted. The last 19 cords were extracted and were numbered 1 through 19. Similar amounts of placental tissue from 12 of the specimens were extracted with water and with saline. Aqueous extracts, only, were made of cords and placentae 15 through 19. An aqueous extract of minced mouse muscle was also prepared as a control.

The extracts were tested in the following ways. Increasing amounts of the extracts were pipetted into small glass vials, the amounts varying from 0.1 cm^3 to 9 cm^3. These extracts were dried at 110°C. One cubic centimeter of freshly drawn human venous blood was then placed in these vials, agitated in order to get as much of the dried extract into solution as possible, and then allowed to stand for one minute. The tubes, including control tubes, were tipped every minute. After 30 minutes, the tubes were tipped every 5 minutes. After two hours, the tubes were watched less closely.

TABLE 1

Total clotting times of blood mixed with aqueous extracts of human umbilical cords

	CONTROL	CONTROL	CORD 2	CORD 3	CORD 4	CORD 5	CORD 6	CORD 7	CORD 8
0.9% NaCl cm³	0	0.1	0	0	0.1	0.1	0.1	0.1	0
Blood cm³	1	1	1	1	1	1	1	1	1
Extract cm³	0	0	2	2					
Cl. T.	9′ 15″	9′ 10″	1 hr. 20′	3 hrs. 15′					
Extract cm³			3	3	3				
Cl. T.			7 hrs. 5′	23 hrs. +	17′ 20″				
Extract cm³					4	4	4	4	
Cl. T.					18′ 35″	12′ 40″	20′ 5″	23′	
Extract cm³			5						5
Cl. T.			17 hrs. +						11′
Extract cm³			6				6	6	6
Cl. T.			17 hrs. +				2 hrs. +	40′ 15″	13′ 40″
Extract cm³			7						
Cl. T.			17 hrs. +						8
Extract cm³			8						13′ 45″
Cl. T.			17 hrs. +		9	9	9	9	9
Extract cm³					19′ 20″	17′ 5″	2 hrs. +	2 hrs. +	16′ 20″
Cl. T.									

Extract (amounts are given in cm³ prior to drying. The extract was tested in the dry state).

TABLE 2

Total clotting times of blood mixed with aqueous and saline extracts of cords

	CONTROL	CONTROL	CORD 9		CORD 10		CORD 11	CORD 12		CORD 13	CORD 14	
			(S.E.)	(H.E.)	(S.E.)	(H.E.)	(H.E.)	(S.E.)	(H.E.)	(H.E.)	(S.E.)	(H.E.)
0.9% NaCl cm³	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Blood cm³	1	1	1	1	1	1	1	1	1	1	1	
Extract cm³	0	0	2		2							
Cl. T.	9′ 15″	9′ 10″	2 hrs. +		2 hrs. +							
Extract cm³											3	3
Cl. T.											2 hrs. +	13′
Extract cm³	0	0	4		4							
Cl. T.	8′ 10″	6′ 30″	2 hrs. +		2 hrs. +							
Extract cm³			6	6	6		6		6	6	6	6
Cl. T.			2 hrs. +	15′	2 hrs. +		63′		21′	2 hrs. +	2 hrs. +	37′
Extract cm³			8		8							
Cl. T.			2 hrs. +		2 hrs. +							
Extract cm³			9	9		9	9	9	9	9	9	9
Cl. T.			2 hrs. +	10′		48′	2 hrs. +	2 hrs. +	63′	2 hrs. +	2 hrs. +	23′

S.E. — Saline extract.
H.E. — Aqueous extract.

It was found with Cord 1 that as little as 2 or 3 cm^3 of the original extract caused marked prolongation of the clotting time. Cord 2 gave similar results. Cord 3 apparently provided a particularly potent extract; 3 cm^3 of dried extract prolonged the clotting time for over 12 hours. Cords 4, 5, 6, 7, and 8 when tested in this manner seemed ineffective, but it was felt that the extract had become too firmly fixed to the sides of the tubes, and that it did not mix with the blood with sufficient rapidity to prevent clotting. For this reason 0.1 cm^3 of 0.9% NaCl solution was placed in each of the tubes containing dried extract and into a control tube, and the anticoagulant activity was retested. The results are given in table 1.

The next 6 cords (9 through 14) were extracted both with saline, as described, and with water. Samples of each showed anticoagulant activity.

Samples of the results obtained are given in table 2. The tubes were watched carefully for only two hours.

Effect of hyaluronidase

Because the umbilical cord is often used as a source of hyaluronic acid, attempts to exclude the possibility that the extracted anticoagulant was hyaluronic acid were made. After 1 cm^3 of blood in the extracts of Cords 3 (4 cm^3), 9 (4 cm^3) [2] and 9 (9 cm^3) [3] had remained fluid for 30 minutes, 0.1 cm^3, 0.2 cm^3 and 0.3 cm^3 of hyaluronidase [4] were added to the three tubes in the order listed. This did not alter the clotting time; all three samples remained fluid longer than two hours. Also 0.3 cm^3 of hyaluronidase had no effect on the clotting time of 1 cm^3 of blood, and 0.5 cm^3 of hyaluronidase had no effect on the clotting time of 3 cm^3 of blood.

Four cubic centimeters of the water extract of Cord 13 was placed in each of two tubes. To one of the tubes, 1 cm^3 of hyaluronidase was added. The tubes remained at room

[2] Saline extract of cord. Results probably not valid.
[3] Saline extract of cord. Results probably not valid.
[4] Hyaluronidase — Wyeth. 150 turbidity units/cm^3.

temperature for 4¾ hours. The extracts were then dried at 110°C. and tested with 1 cm³ of blood, using 0.1 cm³ 0.9% NaCl in each tube to attempt to get the extract into solution. The saline control which lacked both the extract and hyaluronidase clotted in 9′25″. The saline plus extract clotted in 19′30″, and the saline plus extract and hyaluronidase clotted in one hour. A similar test was done with 5 cm³ of the water extract of Cord 11. The saline control clotted in 11′. The saline plus extract clotted in 20′, and the saline plus extract and hyaluronidase clotted in 35′.

Obviously these are only preliminary experiments, but they suggest that the anticoagulant is not destroyed by hyaluronidase. One might wonder if the hyaluronidase liberated more anticoagulant from the viscid extract and thus prolonged the coagulation time. The aqueous extracts in this experiment do not seem sufficiently uniform to warrant this conclusion without further studies.

The effect of hyaluronidase on the mast cells of the smears was also tested. Two dry smears were fixed in Lavdowsky's [5] solution for 15 minutes. One was flooded with saline and the other with 1 cm³ of hyaluronidase. These solutions were left on the slides for one hour. The slides were then stained with Wright's stain. Both showed numerous mast cells which seemed to contain their full component of granules, and the staining reaction of the granules seemed identical in the saline control and in the preparation exposed to hyaluronidase. Two bone marrow smears containing 8 tissue mast cells per 500 nucleated cells (from a patient with aplastic anemia) were subjected to the same treatment as a further check on the method. The mast cells were similarly unaffected by hyaluronidase.

Effect of heat

Because Jacques and Charles ('41) stated that heparin may lose some of its potency on ashing, the effect of heat equal to

[5] Modification of Lavdowsky solution (Williams, Hodge and Wills, '43). Formaldehyde — 10 parts; 95% alcohol — 50 parts; glacial acetic acid — 3.5 parts; distilled water — 40 parts.

that used in drying the extracts of cord was checked by allowing liquid heparin to dry at 110°C. for 6 hours. This treatment did not alter its anticoagulant activity.

Additional controls and experiments

Extracts of minced mouse muscle had no effect on the clotting time. Extracts of placental tissue (8 tested and not included in tables 2 and 3), however, prolonged the clotting time as much as or occasionally more than, extracts of the cords. We have not yet been able to demonstrate significant numbers of tissue mast cells in smears or sections of the placentae. In sections occasional mast cells can be found in the walls of blood vessels.[6]

Blood aspirated from the umbilical cord was mixed with normal blood in dilutions varying from 0.1 to 0.75 cm^3 of cord blood to 1 cm^3 of normal blood. The total clotting time was not altered. Thrombin titrations and prothrombin times were also normal. These experiments as well as the dearth of blood in the cords prior to extraction suggest that cord blood is not the major source of anticoagulant. It should be noted that Piccolo ('50) has commented on the possible presence of an anticoagulant in umbilical cord blood.

Antithrombic activity

Five cubic centimeters of blood was kept fluid by mixing it with 15 cm^3 of dried aqueous extract of Cord 3. Plasma re-

[6] Since this paper was originally presented in abstract (Sundberg, Schaar, Powell and Denboer, '53), tissue mast cells have been described and illustrated in section preparations in the stroma of human chorionic villi by Latta and Beber ('53). They believed that the cells they illustrated were comparable to cells described and illustrated in human placentae by Pescetto ('50). Pescetto found these elements with basophilic granules of a metachromatic type in placentae from the 4th month of pregnancy to the time of gestation. He found these cells in both normal and diseased placentae, but he felt that they were decreased in eclampsia.

moved from this blood prolonged the prothrombin time as follows:

	Prothrombin time
Control	14.1" and 13.3"
Plasma (E. Cord 3)[7]	29.5" – 30.5"

Tests for antithrombic activity of the extract showed the following results:

	Thrombin titration [8]
Control	15.5", 15.9", 16.7", 17"
Plasma (E. Cord 3)	39.7", 37.7", 41.5", 36.2"

Heparinized plasma made up with 0.4 cm^3 of normal plasma and 0.1 cm^3 of a 1/300 dilution of commercial fluid heparin showed thrombin titration times of 42.8", 38.0", and 36.7". This heparinized plasma gave prothrombin times of 15.0", 12.5", and 17.4". When 0.4 cm^3 of normal plasma was combined with 0.05 cm^3 of a 1/150 dilution of heparin and 0.05 cm^3 of a 1/150 dilution of protamine, the thrombin titration times were 18.9", 23.3", 24.4", and 20.9", and the prothrombin times, 12.8" and 15.0". Attempts to neutralize the effect of the anticoagulant in the plasma using varying concentrations of protamine (0.1 cm^3 of protamine 1/1600, 1/400, 1/300, 1/200, and 1/100) were made, but the thrombin titration times (34" to 45") varied only within the range of variability of the times obtained with the plasma alone. Recalcification times showed the following results:

Normal plasma	2' 30"	2' 15"
	2' 15"	2' 00"
Heparinized plasma (described above)	5' 00"	5' 00"
Plasma (E Cord 3)	13' 15"	25' 30"

[7] Plasma obtained from blood mixed with extract of Cord 3.

[8] Technique of thrombin titration: A saline solution of thrombin which will clot normal fresh plasma in approximately 10 seconds is prepared. This concentration is then applied to the unknown. A second thrombin solution of ½ the concentration of the first is prepared and both normal and unknown plasma are tested with this. The time for normals is about 15 seconds with the latter thrombin concentration. The thrombin solution is kept in ice during the procedure to insure maintaining its potency. One-tenth cubic centimeter of plasma is pipetted into a small serological tube; 0.1 cm^3 of the thrombin solution is blown in; and a stop-watch is started simultaneously. Time of clot formation is noted. The test is carried out in a constant temperature water bath at 30°C.

Effect of saline

As can be seen in table 2, saline extracts of the cord apparently contained a powerful anticoagulant. This was true of the corresponding placental extracts as well. Prothrombin time, thrombin titration times and recalcification times were run on plasma obtained from blood mixed with the dried saline extracts of Cord 14 and Placenta 14. Remarkable prolongation of the thrombin titration times and prothrombin times was found. With recalcification, no definite clot formed during the 30 minute period of observation.

The latter observation and the fact that blood mixed with saline extracts of the cords and placentae sometimes remained fluid for several days of observation caused us to be concerned about the possibility of some defect in fibrin formation. It seemed easily possible that the amount of saline in the dried extracts was excessive. Two small vials containing 2 cm^3 and two containing 9 cm^3 of 0.9% NaCl were dried as were the original saline extracts. When 1 cm^3 of blood was added to each of these vials, marked prolongation of the clotting time was obtained. A weak coagulum formed in the vials containing 2 cm^3 of 0.9% NaCl, but nothing identifiable as a clot could be seen in the vials containing 9 cm^3 of 0.9% NaCl. Prothrombin determinations were done on the plasma from each of the tubes with the following results:

	0.1 cm^3 *thromboplastin*	0.1 cm^3 *thromboplastin* 0.1 cm^3 1/80 *M* $CaCl_2$ (Added together)
0.1 cm^3 plasma [from blood mixed with 2 cm^3 of 0.9% NaCl (dried)]	33.8″	19″
0.1 cm^3 plasma [from blood mixed with 9 cm^3 of 0.9% NaCl (dried)]	20′ +	13′

Since dried saline extracts in concentrations similar to those used in extracting the cords and placentae prolonged the clotting and prothrombin times, the results obtained with saline extracts were considered invalid.

TABLE 3

Total clotting times of blood mixed with aqueous extracts of cords and placentae

	CONTROL	15		16		17		18		19	
		C	P	C	P	C	P	C	P	C	P
0.9% NaCl cm³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Blood cm³	1	1	1	1	1	1	1	1	1	1	1
Extract cm³	0		2			2	2	2			
Cl. T.	8′ and 9′		9′			9′	9′	9′			
Extract cm³		4	4				4	4	4		4
Cl. T.		9′	9′				9′	11′	16′		12′
Extract cm³		6	6			6	6	6	6	6	6
Cl. T.		9′	14′			11′	11′	9′	8′	10′	2 hrs.+
		10′	11′								
Extract cm³			8	8	8						
Cl. T.			14′	14′	17′						
				13′							
Extract cm³		9	9	9	9	9	9	9	9	9	9
Cl. T.		13′	15′	10′	12′	25′	28′	19′	2 hrs.+	18′	2 hrs.+
						8′	14′	37′			
							55′				
Extract cm³	0		9			9	9	9	9		9
Cl. T.	9′		25′—			25′+	25′+	1 hr.	25′		2 hrs.+
			25′—			25′+	25′+	1 hr.	1 hr.		2 hrs.+
			25′—			25′+		1 hr.	2 hrs.+		2 hrs.+

C — cord. P — placenta.
Clotting times below the line are those obtained during the third evaluation.
Key: 25′— Blood clotted in centrifuge.
25′ + Blood clotted while thrombin titrations on extracts of C and P 18 and P 19 were being done.
C and P 19 were obtained from a patient who had 102 mg % fibrinogen at the time of delivery. Pregnancy was complicated by abruptio placentae and fetal death.

Anticoagulant activity of cords and placentae 15 through 19

Aqueous extracts of 5 additional cords and placentae were prepared. The results of their effect on the clotting time are shown in table 3. Three separate evaluations were run. Twenty cubic centimeters of blood was drawn each time, and the tubes were filled at random so some of the discrepant results are probably due to the time at which the tubes were filled; the last tubes filled in one of tests, particularly, would not show true values because blood was beginning to coagulate in the syringe even though it took only $3\frac{1}{2}$ minutes to expel the contents of the syringe. In the third evaluation,[9] a siliconized syringe was used, and all the samples tested contained 9 cm^3 of dried aqueous extract.

Thrombin titrations were done on plasma obtained from Cord 18, Placenta 18 and Placenta 19.

Time after blood was drawn	25′	2 hours
Control	15.0″	
Plasma from Cord 18 (1)	14.5″	
(2)	14.5″	
(3)	15.0″	
Plasma from Placenta 18 (1)	15.1″	
(2)	15.4″	
Plasma from Placenta 19 (1)	17.2″	
(2)	16.0″ and 17.4″	19.4″

When thromboplastin only was added to the plasma from Cord 18, no clot occurred in 5′. With the addition of calcium, the prothrombin time was 15″ with a control of 13.5″.

Recalcification of the plasma was done 35 minutes after blood was drawn.

	Recalcification time
Plasma from Cord 18	less than 1′ 30″
Plasma from Placenta 19	2′ 30″

Two hours after the blood was drawn recalcification of plasma obtained from blood mixed with extract of Cord 18 gave a time of 1′18″.

[9] The tests included in the third evaluation were performed by Dr. Paul Frick.

The results obtained on blood mixed with dried aqueous extracts of Cord 18 and Placentae 18 and 19 do not confirm the original results with Cord 3. Since in the last thrombin titration times there seemed to be no significant variation from the normal and since recalcification of the plasma occurred within the time range accepted for oxalated blood, it seems necessary to postulate that the latter extracts probably prolonged coagulation by binding calcium ions rather than through some type of antithrombic activity.

LITERATURE AND DISCUSSION

A review of the phylogenetic and histologic history of histogenous and hematogenous mast cells was provided by Michels ('38). The presence of tissue mast cells in the connective tissues of the liver, lung, subcutaneous tissue, subendothelial tissue, and spleen was emphasized and illustrated by Holmgren and Wilander ('37) and Jorpes, Holmgren and Wilander ('37). Wilander ('38) showed that the amount of heparin extracted from a tissue was roughly proportional to the number of mast cells present, and his experiments give strong support to the hypothesis that tissue mast cells are a source of heparin. Jorpes ('39 and '46) has reviewed the history of the findings of this group of investigators. Tissue mast cells have also been shown to be abundant in human synovial membranes (Davies, '46; Wislocki, Bunting and Dempsey, '47; Janes and McDonald, '48 and Asboe-Hansen, '50). Janes and McDonald treated 10 cm^3 of the 75 cm^3 of bloody fluid which had remained fluid for over two and one-half hours after aspiration from a joint cavity with 10 cm^3 of 1% protamine. Since a coagulum formed with this procedure, they felt that heparin might be partly responsible for the prevention of coagulation in hemarthrosis.

Passarelli ('40) provided two illustrations (figs. 1 and 2) of sections of umbilical cord. In each of these, there are three cells which seem similar to the mast cells found in the present investigation.

In 1950 Asboe-Hansen called attention to the fact that tissue mast cells and metachromatic ground substance are prominent in myxedematous connective tissue. He claimed that hyaluronidase reduced the degree of metachromasia of this tissue. Asboe-Hansen also discussed the fact that Wharton's jelly showed remarkable metachromasia and also large cells with metachromatic cytoplasm and granules. He thought that Wharton's jelly consisted mostly of hyaluronic acid, and that tissue mast cells might be important in the production of hyaluronic acid.

The metachromatic staining reaction of the connective tissue of the umbilical cord was discussed and illustrated in 1947 by Wislocki, Bunting and Dempsey, but these investigators did not describe or illustrate tissue mast cells in the mucous connective tissue of the umbilical cord. They stated that all metachromasia was not removed from the matrix of Wharton's jelly by digestion with hyaluronidase and that mast granules were not digested by hyaluronidase. One might take this to mean that some substance other than hyaluronic acid is responsible for the staining reaction of mast granules and for part of the metachromatic staining reaction present in the connective tissue of the umbilical cord. The present studies on dry smears have confirmed the fact that metachromasia remains in the ground substance and the mast cell granules after digestion with hyaluronidase.

Glick and Sylvén ('51) have shown that tissues rich in mast cells provide a heparin-lipoprotein which is a hyaluronidase inhibitor. In the present studies hyaluronidase did not overcome the antigcoagulant activity of the extracts tested.

Meyer and Rapport ('51) in a review of the mucopolysaccharides of the ground substance of connective tissue have stated that umbilical cord contains hyaluronic acid and chondroitin sulfate C both of which are hydrolyzed by hyaluronidase. They mention that heparin might be a mucopolysaccharide occurring in ground substances, but that with their present methods they have not yet encountered it.

Snellman, Sylvén and Julén ('51) have extracted and purified a native heparin-containing compound or native antithrombic material from tissue mast cells of ox liver capsules or rat skin. This material was shown to contain heparin associated with a polypeptide and a lipid residue. They showed that this native heparin complex, a "rapidly water-soluble microsome material" has a greater anticoagulant activity than corresponding amounts of purified heparin. They felt that from a cytochemical point of view, their results suggested that the whole heparin complex is probably produced in the intergranular cytoplasm of the tissue mast cells.

Although the present studies regarding the anticoagulant in extracts of umbilical cord were made on a very crude extract, it was hoped that the material extracted might prove similar to heparin or to the native heparin complex. Antithrombic activity comparable to that shown in plasma mixed with aqueous extracts of Cord 3 was not demonstrated in aqueous extracts of Cord 18 and Placentae 18 and 19, and the addition of calcium corrected the clotting defect. Also the antithrombic activity of Cord 3 was not neutralized with concentrations of protamine sufficient to neutralize equally antithrombic solutions of heparin. We do not understand the discrepancy in antithrombic activity between Cord 3 and Cord 18 and Placentae 18 and 19.

Shulman and Ferry ('50) have shown that the clotting time of bovine fibrinogen and thrombin solutions increases as the ionic strength of a sodium chloride solution increases and that clotting also varies with pH. These results were confirmed by Edsall and Lever ('51). The known high concentration of sodium chloride in the saline extracts in the present investigation would account for much of the marked prolongation in the clotting times, prothrombin times, and thrombin titrations even without the addition of whatever substance was extracted from the cords and placentae.

Koller ('52) in a review of the physiology and pathology of blood coagulation summarized two interesting experiments of Rovatti. Rovatti ('51a) showed that in experimental ani-

mals intraperitoneal injections of heparin resulted in a temporary metachromatic coloring of the liver tissue, and in the appearance of numerous metachromatic granules in the cells of the walls of the small vessels. After injection of peptone solution the metachromic granules in the cells in the area of the intima of the vessels disappeared. Rovatti ('51b) also claimed that the injection of hyaluronidase into experimental animals which previously had been prepared with heparin led to a rapid disappearance of the metachromatic coloring of the liver tissue and of the basophil granules of the mast cells. The latter finding surely deserves further investigation.

Compton ('52), in a study which dealt largely with the mast cells of the hamster, pointed out that the distribution of mast cells within the hamster does not support their supposed function as "heparinocytes." He emphasized the fact that the metachromasia indicates that they contain a compound with high molecular weight and an acidic function, but that this compound has not yet been proven to be heparin. His experiments with hyaluronidase caused him to conclude that the hamster mast cell granules do not include hyaluronic acid.

Since some of the results of the present investigation appeared in abstract (Sundberg, Schaar, Powell and Denboer, '53), Martin and Roka ('53) have reported the isolation of a substance which inhibits coagulation from the leukocytes of a patient with "mast cell leukemia." The substance was "heat stable, acid-fast and water-soluble and inhibited coagulation possibly by opposing thrombokinase. Its biological and physico-chemical properties are the same of those of heparin." Unpublished experiments (Sundberg and Powell) on aqueous extracts of leukocytes in patients with chronic myelogenous leukemia and a very high percentage of basophils have shown the presence of some type of anticoagulant in the leukocytes of the clot from as little as 5 cm^3 of blood. These extracts showed some prolongation of the thrombin titration but recalcification brought about clot formation in $3\frac{1}{2}$ minutes. The

experiments of Martin and Roka are of extreme interest, and surely further work should be done on this subject.

SUMMARY AND CONCLUSIONS

1. Wharton's jelly of the human umbilical cord contains numerous tissue mast cells. Fibroblasts and other connective tissue cells containing metachromatic granules are also abundant. It is postulated that the latter cells may be precursors of or may actually be tissue mast cells. All of these cell types have been demonstrated in dry smears and sections of the umbilical cord.

2. The staining reaction of the mast granules in dry smears of Wharton's jelly or bone marrow was not altered by hyaluronidase.

3. Aqueous or saline extracts of the umbilical cord and of the placenta have anticoagulant activity. The concentration of sodium chloride in the saline extracts was found to be sufficiently great to inhibit coagulation, and, therefore, the results obtained with the saline extracts are not considered valid.

4. Preliminary experiments with hyaluronidase suggest that it does not destroy the anticoagulant.

5. Initial specific tests on the aqueous extract of one of the cords suggested that the anticoagulant was an antithrombin. Later tests on the aqueous extracts of one cord and two placentae showed no significant antithrombic effect, and coagulation occurred promptly after the addition of calcium. The latter results suggest that clotting was probably inhibited by the binding of calcium, but we have no explanation for the initial finding of an antithrombic substance. The initial antithrombic substance was not, however, neutralized with dilutions of protamine that were adequate to neutralize equally antithrombic solutions of heparin.

LITERATURE CITED

ASBOE-HANSEN, G. J. 1950 The intercellular substance of the connective tissue in myxedema. J. Invest. Dermat., *15:* 25–32.

COMPTON, A. S. 1952 A cytochemical and cytological study of the connective tissue mast cell. Am. J. Anat., *91:* 301–329.

DAVIES, D. V. 1946 Synovial membrane and synovial fluid of joints. Lancet, *251:* 815–819.

DOWNEY, H. 1913 The development of histogenous mast cells of adult guinea pig and cat, and the structure of the histogenous mast cells of man. Folia Haemat., *16:* 49–74.

EDSALL, J. T., AND W. F. LEVER 1951 Effects of ions and neutral molecules on fibrin clotting. J. Biol. Chem., *191:* 735–756.

GLICK, D., AND B. SYLVÉN 1951 Evidence for the heparin nature of the non-specific hyaluronidase inhibitor in tissue extracts and blood serum. Science, *113:* 388–389.

HOLMGREN, H., AND O. WILANDER 1937 Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen. Zeitschr. f. mikr. anat. Forschung, *42:* 242–278.

JACQUES, L. B., AND A. F. CHARLES 1941 The assay of heparin. Quart. J. Pharm. and Pharmacol., *14:* 1–15.

JANES, J., AND J. R. MCDONALD 1948 Mast cells. Their distribution in various human tissues. Arch. Path., *45:* 622–634.

JORPES, J. E. 1939 Heparin. Oxford University Press, London. 86 pp.

———— 1946 Ibid. Ed. 2. Oxford University Press, London. 260 pp.

JORPES, E., H. HOLMGREN AND O. WILANDER 1937 Über das Vorkommen von Heparin in den Gefässwanden und in den Augen. Zeitschr. f. mikr. anat. Forschung, *42:* 279–301.

KOLLER, F. 1952 Physiology and pathology of blood coagulation. Acta haemat., *8:* 329.

LATTA, J. S., AND C. R. BEBER 1953 Cells with metachromatic cytoplasmic granules in the stroma of human chorionic villi. Science, *117:* 498–499.

LEHNER, J. 1924 Das Mastzellen-Problem und die Metachromasie-Frage. Ergebn. d. Anat. u. Entwgsch., *25:* 67–184.

MARTIN, H., AND L. ROKA 1953 Zur Frage des Heparin-Gehaltes der Blutmastzellen des Menschen. Acta haemat., *10:* 26–31.

MAXIMOW, A., AND W. BLOOM 1952 Textbook of Histology. Ed. 6. Saunders, Philadelphia. 616 pp.

MEYER, K., AND M. M. RAPPORT 1951 The mucopolysaccharides of the ground substance of connective tissue. Science, *113:* 596–599.

MICHELS, N. A. 1938 The mast cells. In Downey, H.: Handbook of Hematology. Hoeber, New York. Vol. I, Sect. IV, pp. 232–372.

PASSARELLI, G. 1940 Mastzellen e cellule tiazinofile nelle condisioni normali e patologiche dell'apparato genitale femminile umano. Rivista di Biol., *30:* 93–113.

PESCETTO, G. 1950 Sulla presenza di elementi granulosi basofili metacromatici nella placenta fetale umana. Biologica Latina, *2:* 744–757.

PICCOLI, R. 1950 L'attivita' eparinica del sangue del cordone ombelicale. Bull. Soc. Ital. Biol. Sper., *26:* 623–625.

ROVATTI, B. 1951a Comportamento delle mastzellen nello shock da peptone. Boll. Soc. med. chir. Pavia, *65:* 621. Cited from Koller.

———— 1951b Azione della jaluronidasi sulle mastzellen e sugli endoteli dei vasi dopo carico di eparina. Ibid., *65:* 635. Cited from Koller.

SHULMAN, S., AND J. D. FERRY 1950 The conversion of fibrinogen to fibrin. II. Influence of pH and ionic strength on clotting time and clot opacity. J. Phys. and Colloid Chem., *50:* 66–79.

SNELLMAN, O., B. SYLVÉN AND C. JULÉN 1951 Analysis of the native heparin-lipoprotein complex including the identification of a heparin complement (heparin co-factor) obtained from extracts of tissue mast cells. Biochim. et Biophys. Acta, *7:* 98–109.

SUNDBERG, R. D., F. E. SCHAAR, M. J. S. POWELL AND D. DENBOER 1953 Tissue mast cells in human umbilical cord, and the anticoagulant activity of dried extracts of cords and placentae. Anat. Rec., *115:* 372.

WILANDER, O. 1938 Studien über Heparin. Skand. Arch. Physiol., *81.* Suppl. 15. 89 pp.

WILLIAMS, W. L., H. C. HODGE AND J. H. WILLS 1943 Intravitam staining and toxicity of chlorazol fast pink in mice and rats. Anat. Rec., *87:* 181–206.

WISLOCKI, G. B., H. BUNTING AND E. W. DEMPSEY 1947 Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. Am. J. Anat., *81:* 1–37.

ZOLLINGER, H. U. 1950a Gewebsmastzellen und Heparin (Phasenmikroskopische Untersuchung). Experientia, *6:* 384–390.

———— 1950b Les mitochondries (Leur étude à l'aide du microscope à contraste de phases). Rev. d'Hémat., *5:* 696–745.

PLATE 1

EXPLANATION OF FIGURES

All figures are photomicrographs of cells from dry smear preparations of Wharton's jelly from human umbilical cord.

1 Three tissue mast cells × 250. Wright's stain.

2 Tissue mast cell × 1400. Stained with toluidine blue.

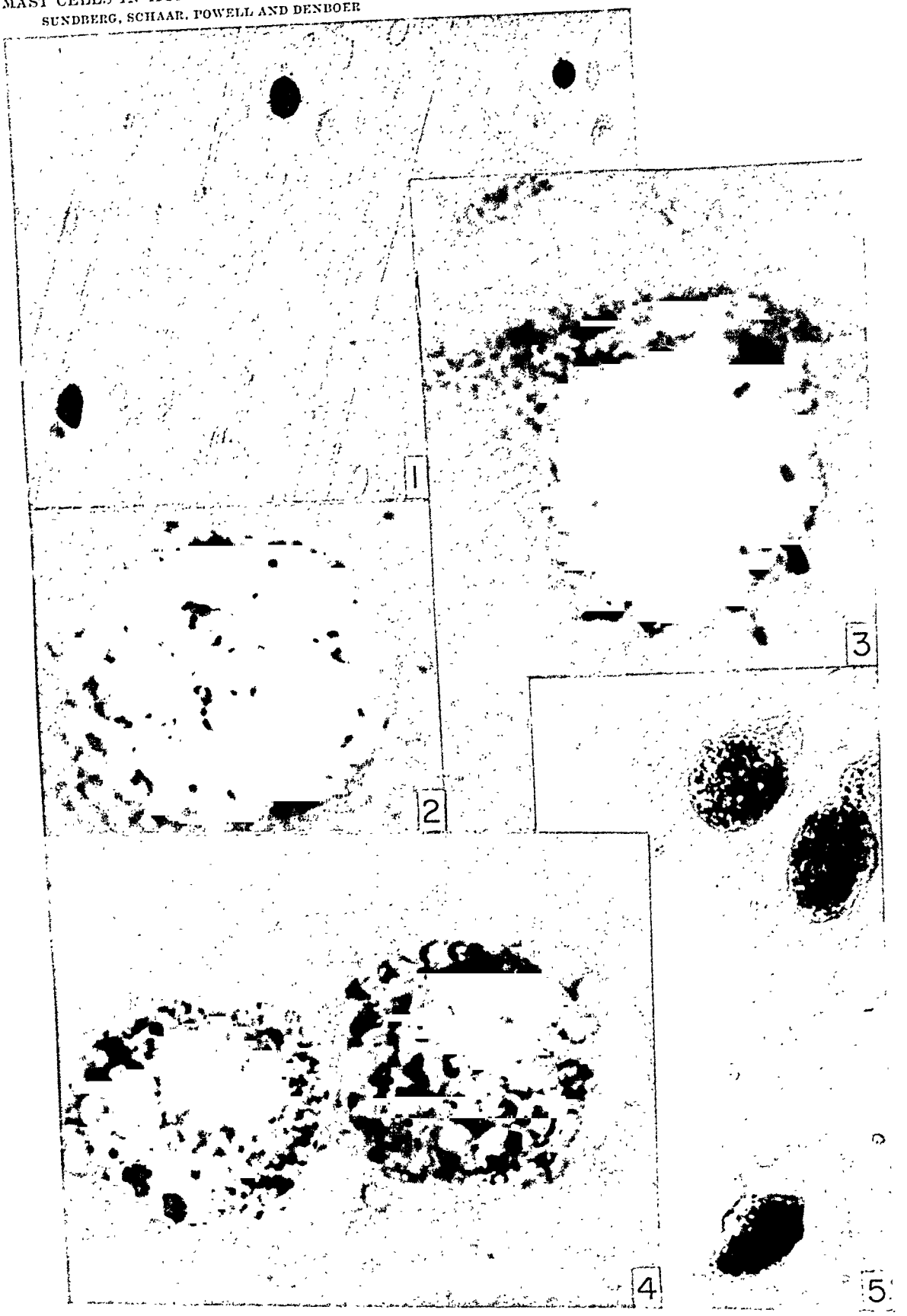
3 Tissue mast cell × 1400 with metachromatic granules which vary remarkably in size. Wright's stain.

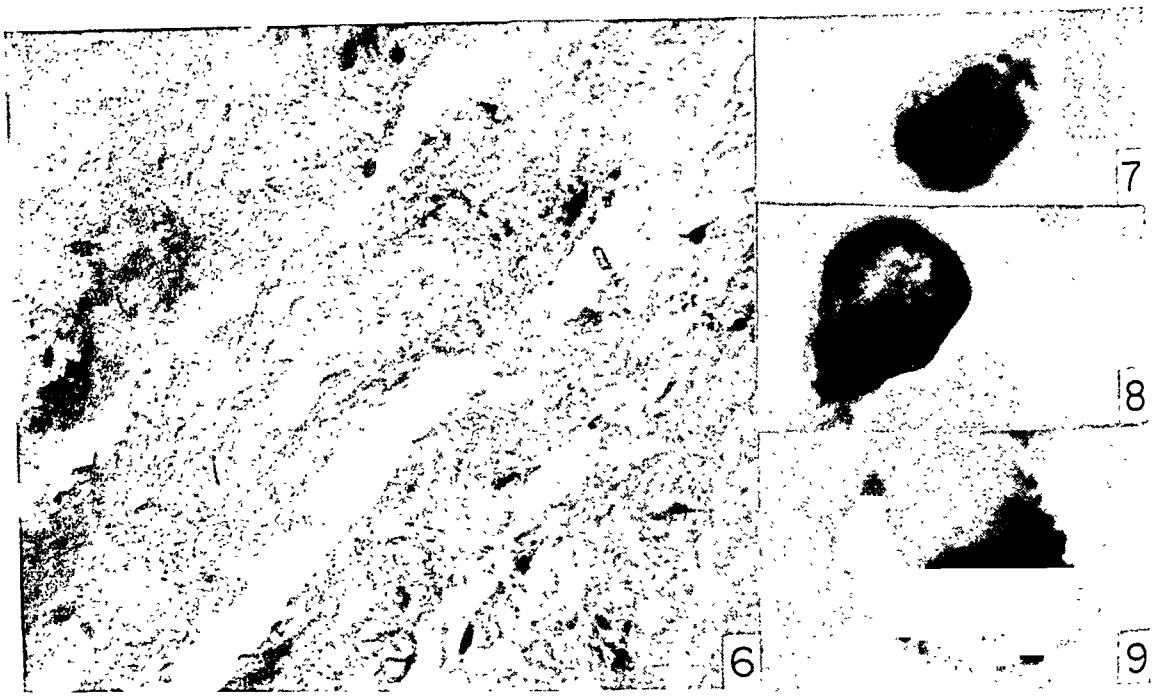
4 Two connective tissue cells × 1200 with relatively abundant cytoplasm showing metachromatic granules of varying sizes and shapes and some vacuolization of the cytoplasm. Possible mast cells. Wright's stain.

5 Three cells similar to those in figure 4 × 750. Wright's stain.

MAST CELLS IN HUMAN UMBILICAL CORD

SUNDBERG, SCHAAR, POWELL AND DENBOER





Photomicrographs of section preparations of Wharton's jelly from human umbilical cord. Fixative — 8% basic lead acetate. Stain — toluidine blue.

6 Section of Wharton's jelly from umbilical cord × 250. The small black structures are tissue mast cells.

7 Tissue mast cell × 1250. This cell contains several vacuoles and 8 or 9 barely visible granules. This cell can be located just above the central portion of the field in figure 6.

8 Tissue mast cell × 1250. The light area is nucleus. Granules can be seen in the surrounding cytoplasm and in the cytoplasm overlying the nucleus.

9 Tissue mast cell × 1400. The light area is nucleus. Granules of varying sizes are clearly visible in the surrounding cytoplasm.

ELECTRON MICROSCOPIC OBSERVATIONS OF PULMONARY ALVEOLI

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EIGHT FIGURES

INTRODUCTION

The morphology of the pulmonary alveolar walls, where the respiratory capillaries and the air spaces are juxtaposed, has been a subject of scientific controversy since the work of Reisseisen (1808), Elenz (1864), and Koelliker (1881). Reisseisen believed that the air cells were merely blind extremities of as many bronchial tubes lined by ordinary mucous membrane. Elenz and Koelliker maintained that a continuous membrane lined the alveolar wall, separating the capillary endothelium from the air spaces. These investigators further maintained that the membrane was composed of nucleated epithelial cells and large, non-nucleated plaques. Earlier workers, Addison (1841) and Williams (1855), held similar views. Koelliker's description however was universally accepted, essentially ending all investigation of the alveolar membrane. His illustrations were frequently reproduced and his authority was sufficient to silence doubters until Miller ('32) reopened the issue.

The thesis of an alveolar membrane composed of nucleated epithelial cells and large non-nucleated plaques is supported by such recent investigators as Bensley and Bensley ('35), Macklin ('36) and Miller ('32). Low ('52) maintains that the alveoli are lined by attenuated epithelial cytoplasm but denies the existence of non-nucleated plaques. Supporting the thesis that the respiratory capillaries are bare except for

supporting reticular fibers and connective tissue ground substance are Loosli, Adams and Thornton ('49) and Potter and Loosli ('51).

A limiting factor preventing a final settlement of alveolar morphology by earlier investigators is the insufficient resolving power of the light microscope. The tissue covering the respiratory capillaries is so sparse that its visualization is accomplished only at the limit of the resolving power of the light microscope. The purpose of this paper is to analyze the alveolar membrane aided by the greater resolving power and magnification of the electron microscope. In this study it was necessary only to utilize the lower limits of the electron microscope's magnification. Recourse to the practical maximum magnification employed by virologists and ultra-morphologists is unnecessary and unrewarding.

MATERIALS AND METHODS

Normal healthy adult female white rat lungs were used for this study. The animals were anesthetized with ether. Working as rapidly as possible while the heart was still beating, perfusion was performed through the right ventricle, first with physiological saline solution and then with 1% osmic acid buffered by veronal acetate at a pH ranging from 7.5 to 8.0 (Palade, '52). The lungs and heart were removed en bloc. The lungs were inspected grossly for any manifestations of pulmonary pathology. Tissue slices not exceeding 1 mm square were excised from those lung areas which were most darkened by the perfusate. These sections were then immersed 8 hours in the same fixative. The tissues were washed overnight in running tap water, dehydrated for one hour by passage through the alcohol series and embedded in n-butyl methacrylate in no. 4 gelatin capsules by heating at 45°C. overnight. The embedding material was catalyzed with 2-4-Dichlorobenzoyl Peroxide and was prepolymerized to a viscous state by heating for two to 4 hours at 45°C. before introduction of the tissue. Embedded tissues were cut on an International Minot

Rotary Microtome using a glass knife set for 1/20 μ (Latta and Hartmann, '49).

Efforts were made to cut tissues as close to .05 μ as possible. Sections were mounted on formvar coated nickel grids for study and photographing on an RCA EMU model electron microscope.

RESULTS

Every section examined shows the existence of tissue covering the respiratory capillaries. The interalveolar septa are seen to contain two types of cells, viz., endothelial cells (figs. 1, 3, 4, 5, 6) and epithelioid cells (fig. 4), modifications of which are designated septal cell (figs. 2, 5) (Lang, '25).

Endothelial cells are identifiable by their oval nuclei and their cytoplasmic processes which extend from the nuclei to form the walls of the septal capillaries (figs. 1, 3, 5, 6). Centrally, the septa are composed of a homogeneous material containing scattered particles of high electron density together with fine electron dense lines of varying length. The septal wall ranges from 1.9 μ to 10.2 μ in width, with an average thickness of 4.8 μ.

The septal wall material has the appearance of a reticulum and seems to serve as a supporting framework for the capillaries. Septal material is seen extending over the capillaries, intervening between their walls and the alveolar spaces (figs. 2, 3, 7). In some instances this substance forms a discrete membrane lining the alveoli (figs. 1, 5, 6). In other places it appears to be so thin and closely adherent to the capillary walls that unless one examines the electron micrographs critically, the capillaries may be interpreted as lying in direct contact with the alveolar spaces (figs. 2, 3, 7). If one traces the apparently naked capillary, a measurable thickness of endothelial tissue is soon found where it turns toward the interior of the septum (figs. 3, 5), becoming separated from its fine covering "membrane." Every section shows at some point this intimate relationship of the endothelial cytoplasm and the membrane-like structure covering it. The transition is

gradual and reaches its maximum thickness as it approaches the nucleus (fig. 1). In a number of illustrations there is a variable amount of intercellular substance between the endothelium and the alveolar space (figs. 5, 7). This intercellular material does not resemble the cytoplasm of the endothelial cell, but in the electron micrograph is seen to contain discrete, fine-lined fibrillar material.

Septal cells are sparse compared to the number of endothelial cells. They are seen as large irregularly-shaped cells which lie in intimate association with the septal stroma. The septal cell cytoplasm is vacuolated and may occupy the entire width of the alveolar wall projecting into adjacent alveolar spaces (fig. 5).

Occasional sections studied show free macrophages in the alveoli (fig. 8). Morphologically, these macrophages resemble septal cells. However, the macrophages contain a large amount of intracytoplasmic electron-dense material.

The endothelial cell is observed in certain areas to separate from the alveolar wall (fig. 1). This physical separation may be due to artifact or to chance. The duality of membranes formed by the separation (fig. 1) serves to illustrate the attenuation of the endothelial cytoplasm. This cytoplasm is drawn out in a fine thin line with stromal material separating it from the air space (fig. 6). But in no case were we able to observe that the cytoplasm of the endothelial cell faced directly on the air space. The alveolar septal material intervenes between the air spaces and the capillary endothelium.

DISCUSSION

Special efforts were made in this investigation to study and photograph only those alveolar walls of smallest diameter. Those parts of the bronchial tree which are higher up and of relatively wider diameter are known to contain a distinct epithelial layer. Concentration on or selective study of these larger sections of the bronchial apparatus can lead to the interpretation of a distinct alveolar epithelium. It is clear that

some material intervenes between the air space and the capillary. The evidence presented in this paper indicates that this material is a continuation of the alveolar septal stroma. No evidence for the presence of a continuous cytoplasmic attenuation of an epithelial cell as described by Low ('52) was found.

Downgrowth of epithelial cells to cover the interalveolar septa can occur in certain pathological conditions. However, no valid evidence can be offered in support of its normal occurrence. Such cellular downgrowth is suggested as the mechanism of the marked epitheliation of alveoli observed in jaagsiekte, a disease of sheep, and found by Bonne ('39) and Bell ('43) to occur in man. The pathogenesis of certain alveolar cell tumors is explained also as a rapid and irregular downgrowth of bronchial epithelium. Grady and Stewart ('40) produced alveolar carcinomas in strain A mice by subcutaneous injections of methylcholanthrene. Whether these tumors had their origin in the alveolus or whether they represent "downgrowths" is not known, but it is hazardous to reason from the abnormal to the normal. The genesis of alveolar epithelium as a result of certain pathodynamic factors does not establish proof of its normal existence.

The central stroma does not resemble either the endothelial or the epithelial cytoplasm. Instead this stroma is composed of ground substance often containing fibrillar material, which is seen in the electron micrograph as a parallel series of fine electron-dense lines (figs. 2, 3, 5, 7). This structure is consistent in appearance with the reticulum observed by Loosli and associates ('49) in silver-stained preparations. The thickness of this material is extremely variable and irregular when seen in section.

If the material which intervenes between the alveolar spaces and septal capillaries is a continuation of the central stroma, the question may be raised as to why this material facing the capillary gives, in certain situations, the appearance of a discrete membrane similar to the capillary wall. It might be postulated that the membrane-like appearance results from

pressure and inflationary factors which are operative throughout life in the postnatal lung.

There is no evidence in our study to suggest that the septal cells are phagocytic. The free macrophages which resemble septal cells are seen to contain a large amount of electron-dense intracellular material. This material may be particulate and it is quite likely that these cells have a phagocytic function. Leucocytes (figs. 2, 7) and erythrocytes (fig. 3) have an appearance in the electron micrograph very similar to their appearance under the light microscope and are readily identifiable.

SUMMARY AND CONCLUSIONS

1. Sections of rat lung were studied by standard procedures under the electron microscope.

2. There is septal material intervening between the capillary and pulmonary alveolus.

3. Evidence for the existence of a continuous epithelial lining between the alveolus and capillary is lacking.

4. Evidence for the existence of non-nucleated plaques is lacking.

5. Other alveolar cells are described.

LITERATURE CITED

ADDISON, W. 1841 Observations on the anatomy of the lungs. Medico Chir. Trans.

BELL, E. T. 1943 Hyperplasia of pulmonary alveolar epithelium in disease. Am. J. Path., *19:* 901–911.

BENSLEY, R. D., AND S. H. BENSLEY 1935 Studies of lining of pulmonary alveolus of normal lungs of adult animals. Anat. Rec., *64:* 41–49.

BONNE, J. 1939 Morphological resemblance of pulmonary adenomatosis (jaagsiekte) in sheep and certain cases of cancer of lung in man. Am. J. Cancer, *35:* 491–501.

ELENZ, E. 1863 Ueber das Lungenepithel. Würzburg. naturwiss. Z., *5:* 66–97.

GRADY, H. G., AND H. L. STEWART 1940 Histogenesis of induced pulmonary tumors in strain A mice. Am. J. Path., *16:* 417–432.

KÖLLIKER, A. 1881 Zur Kenntnis des Baues der Lunge des Menschen. Verhandl. d. Phys.-med. Gesellsch. in Würzb., *16:* 1–24.

LATTA, H., AND J. F. HARTMANN 1950 Use of a glass edge in thin sectioning for electron microscopy. Proc. Soc. exp. Biol., *74:* 436–439.

LOOSLI, C. G., W. E. ADAMS AND T. M. THORTON 1949 The histology of the dog's lung following experimental collapse; with special reference to the nature of the alveolar lining. Anat. Rec., *105:* 697–721.

LOW, F. N. 1952 Electron microscopy of the rat lung. Anat. Rec., *113:* 433–449.

MACKLIN, C. C. 1936 Pulmonic alveolar epithelium; report of round table conference. J. Thoracic Surg., *6:* 82–88.

MILLER, W. S. 1947 The Lung. C. C Thomas, Springfield, Ill., 2nd ed.

PALADE, G. E. 1952 Study of fixation for electron microscopy. J. Exp. Med., *95:* 285–298.

POTTER, E. L., AND C. G. LOOSLI 1951 Prenatal development of the human lung. AMA Am. J. Dis. Child., *82:* 226–228.

REISSEISEN, F. D. 1808 Ueber die Struktur, die Verrichtung, und den Gebrauch der Lungen. Berlin.

WILLIAMS, T. 1855 Epithelium of the air cells of the human lungs. Med. Times and Gaz., *11:* 361–384.

PLATES

Abbreviations

ALV, pulmonary alveolus	G, nickel grid
ADC, alveolar dust cell	L, leucocyte
END, nucleus of endothelial cell	S, septal cell
E, erythrocyte	St, stroma
EP, epithelioid cell	CAP, capillary

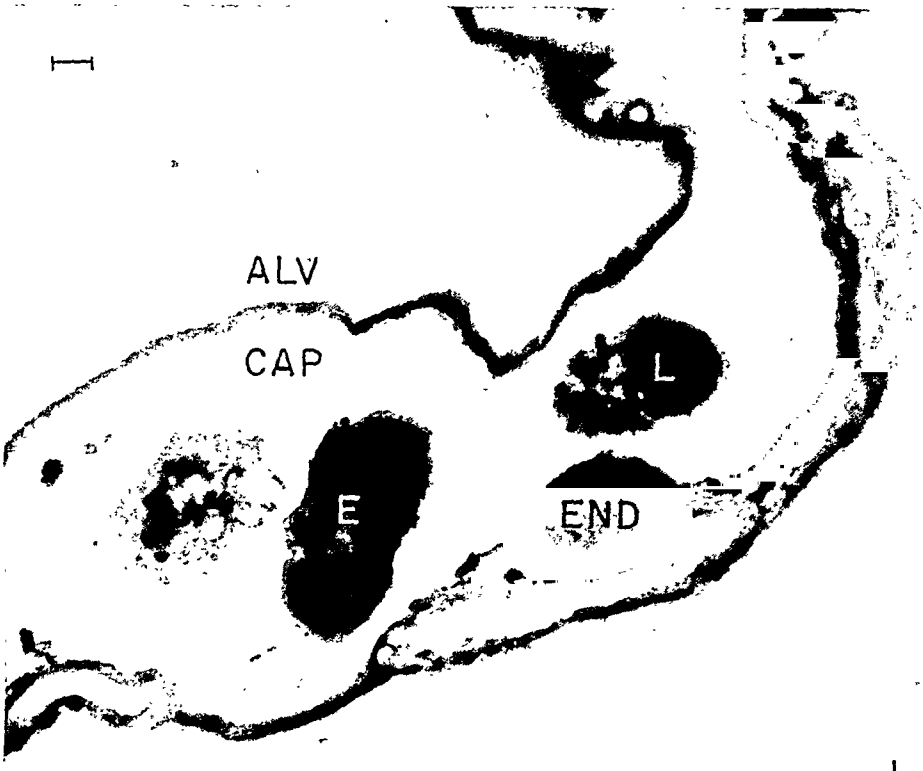
Bar on electron micrograph is equivalent to 1 μ.

PLATE 1

EXPLANATION OF FIGURES

1 Section through alveolus showing endothelial nucleus separated from alveolar lining. Note cytoplasmic attenuation of endothelial cell. 4500 ×.

2 Section showing septal cell. Note alveolar lining on both sides of capillary. 7800 ×.



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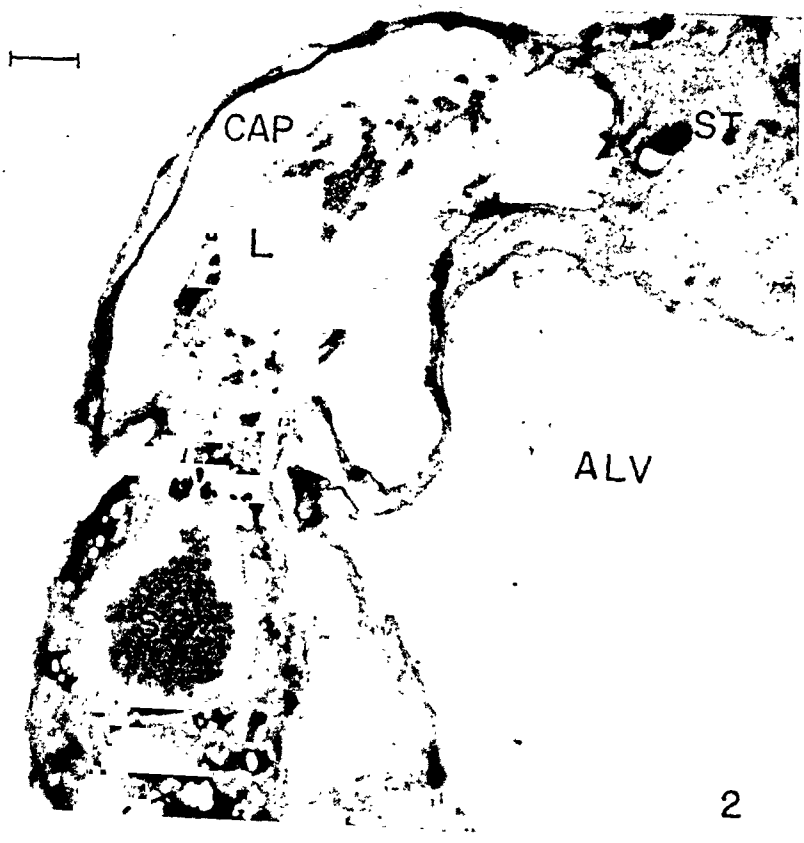

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PLATE 2

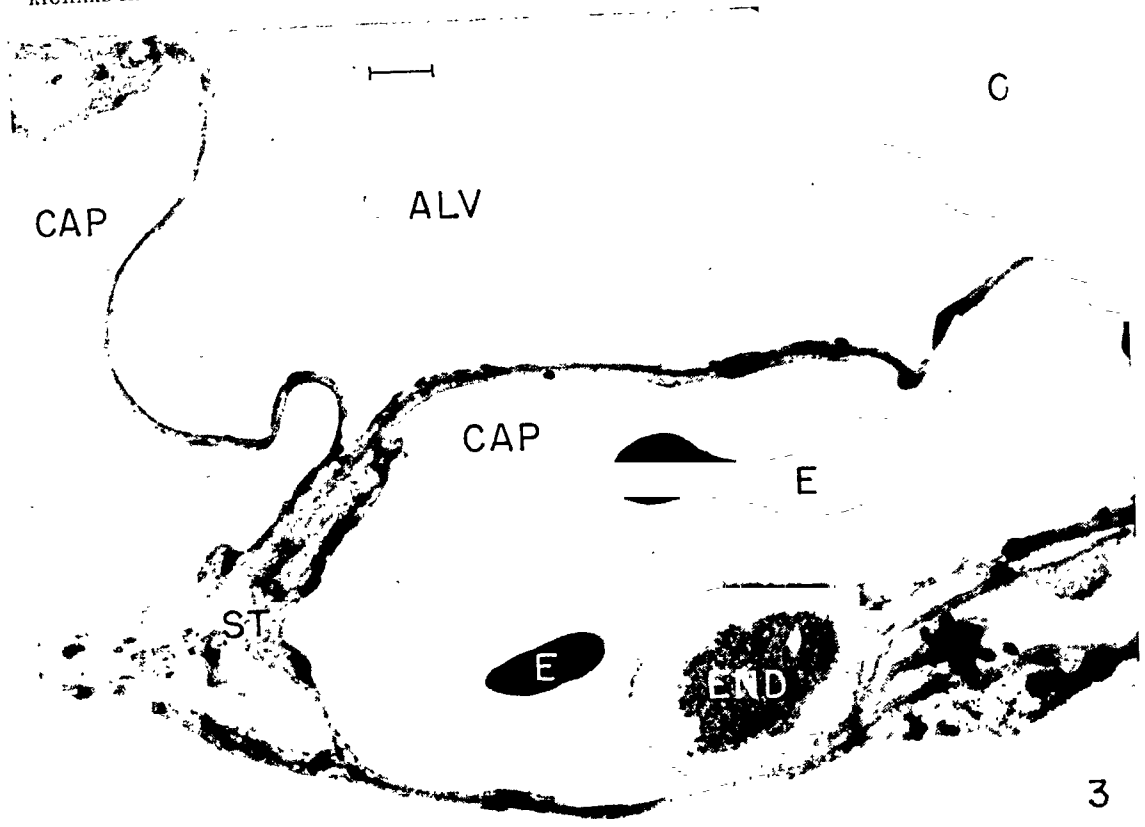
EXPLANATION OF FIGURES

3 Alveolar lining appears as extension or continuation of stroma situated between capillaries. 6900 ×.

4 Section through two capillaries. Epithelioid cell is obvious, but evidence for cytoplasmic attenuation is lacking. 4500 ×.

STRUCTURE OF PULMONARY ALVEOLI

RICHARD H. SWIGART AND DENNIS J. KANE



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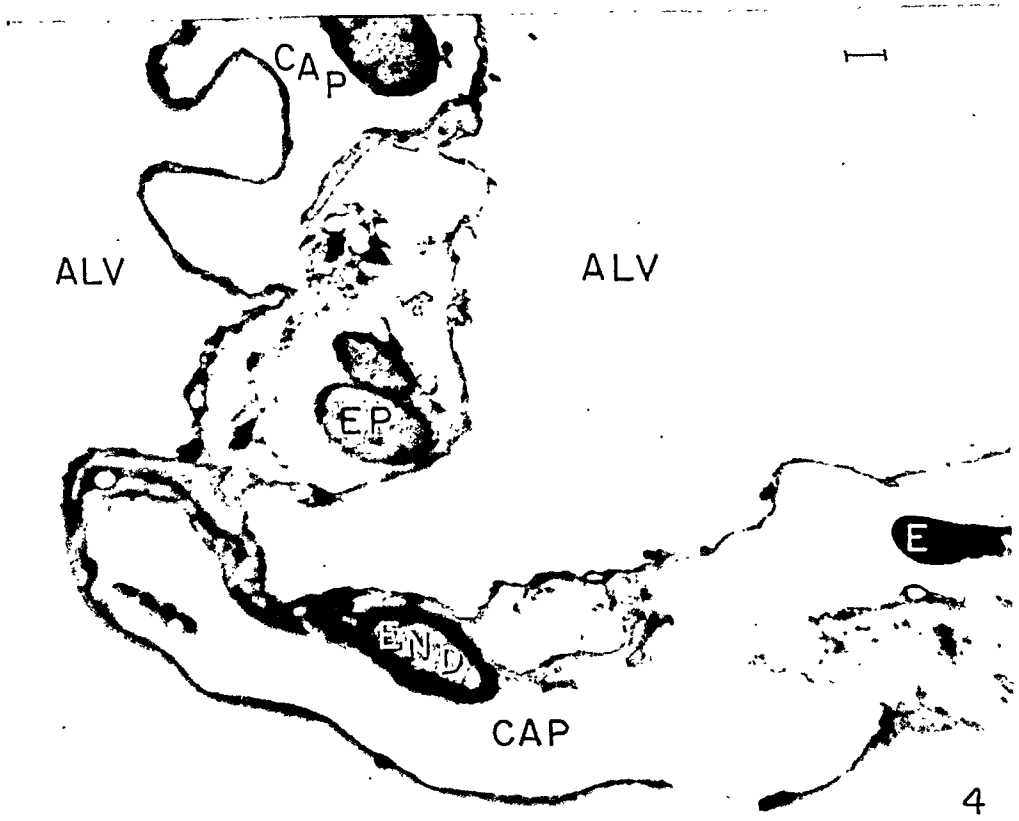

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PLATE 2

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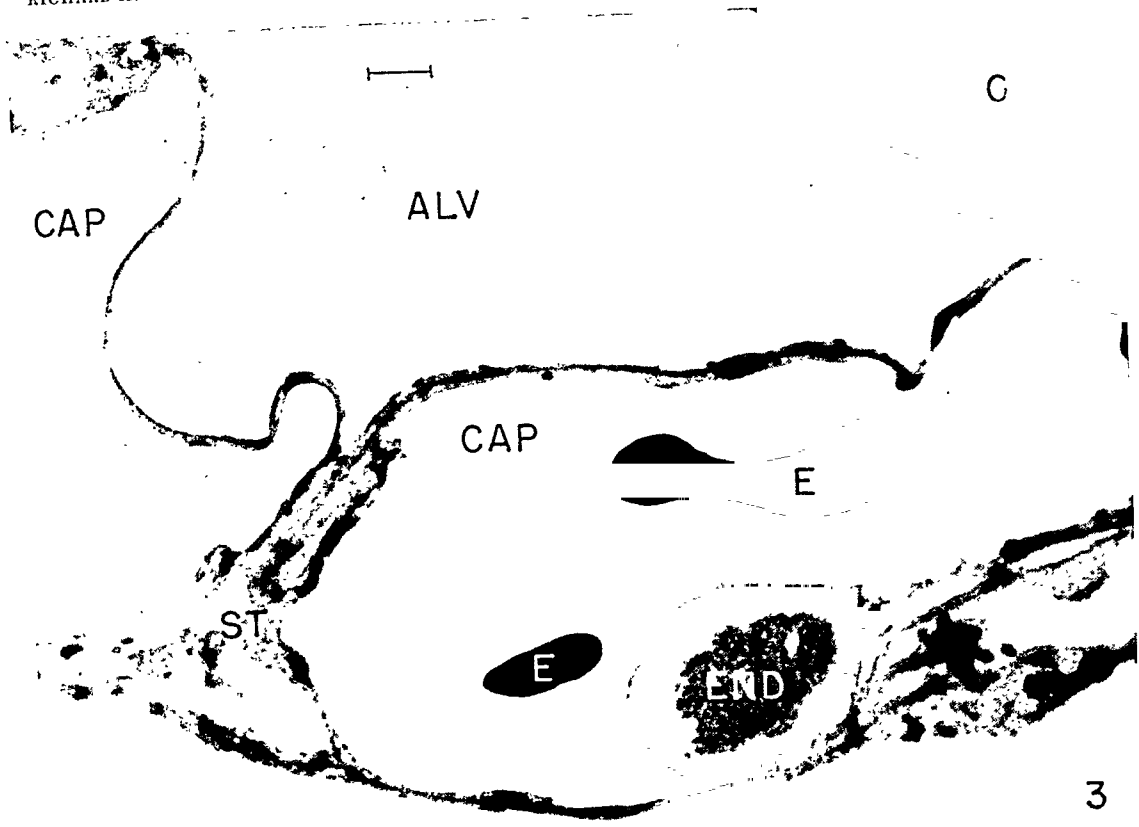
STRUCTURE OF PULMONARY ALVEOLI

RICHARD H. SWIGART AND DENNIS J. KANE



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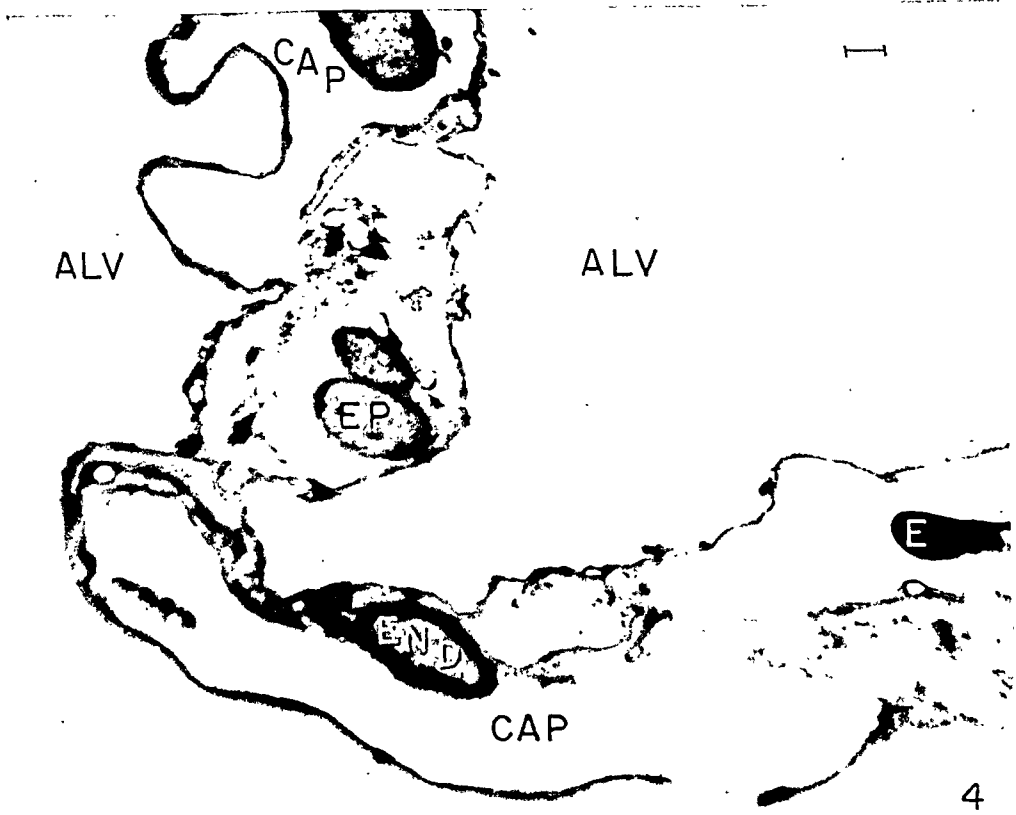

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PLATE 3

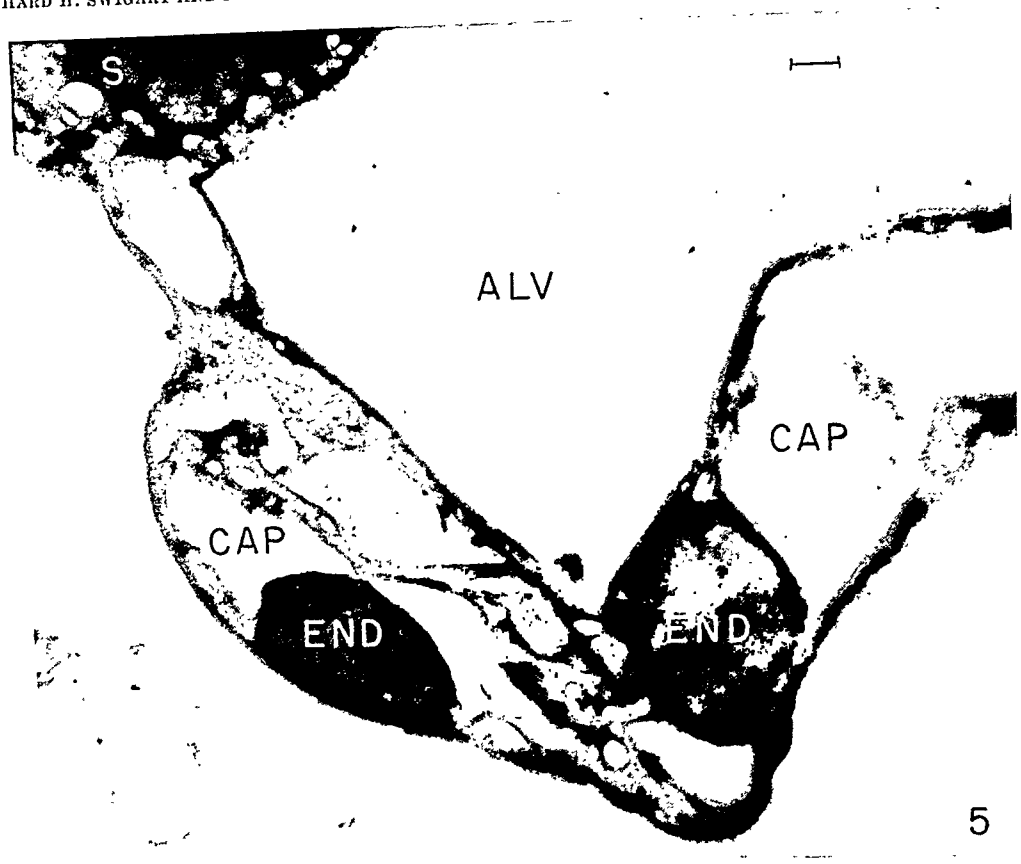
EXPLANATION OF FIGURES

5 Section demonstrating septal cell and two capillaries, each showing endothelial nucleus. 5300 ×.

6 Capillary showing blood cells and endothelial nucleus. 6600 ×.

STRUCTURE OF PULMONARY ALVEOLI

RICHARD H. SWIGART AND DENNIS J. KANE



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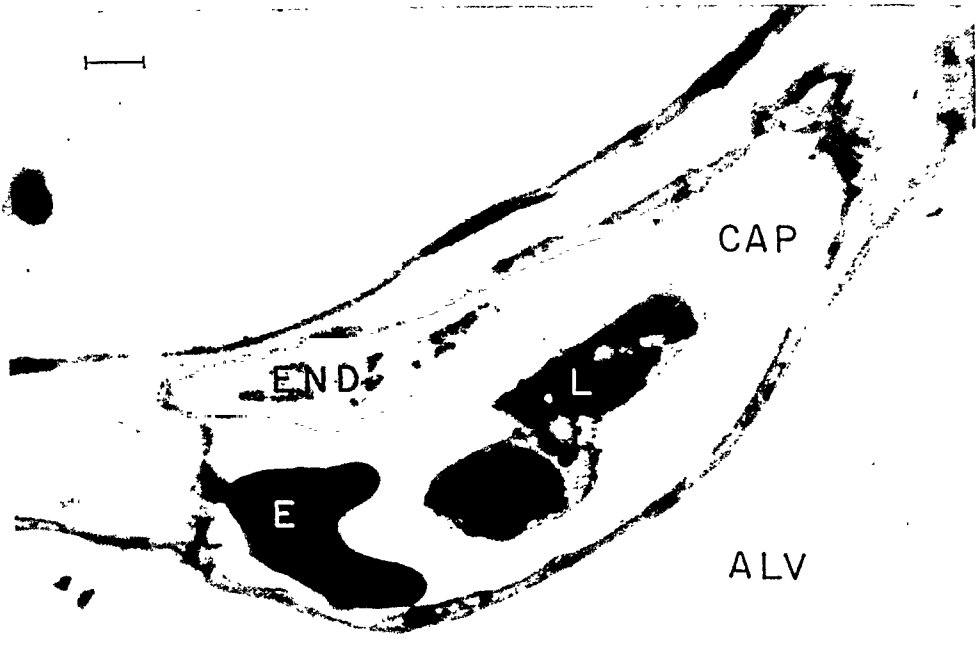

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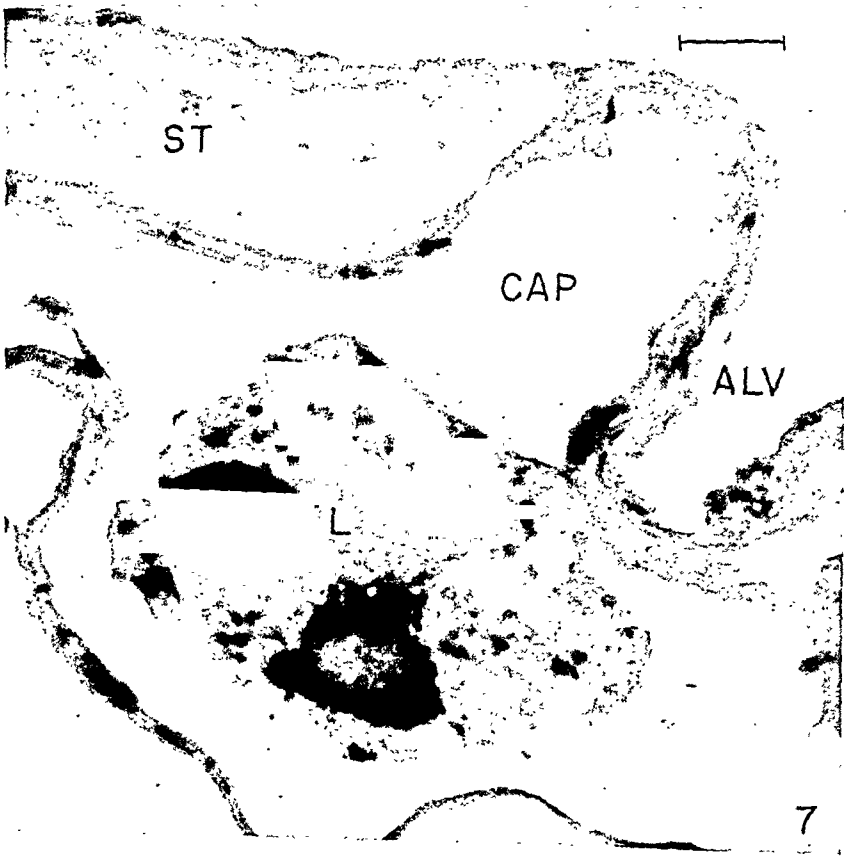
PLATE 4

EXPLANATION OF FIGURES

7 Note interposition of stroma between capillary and alveolar space. 11,500 ×.

8 Section showing alveolar dust cell free in pulmonary alveolus. Alveolar wall structures are considerably distorted. 4500 ×.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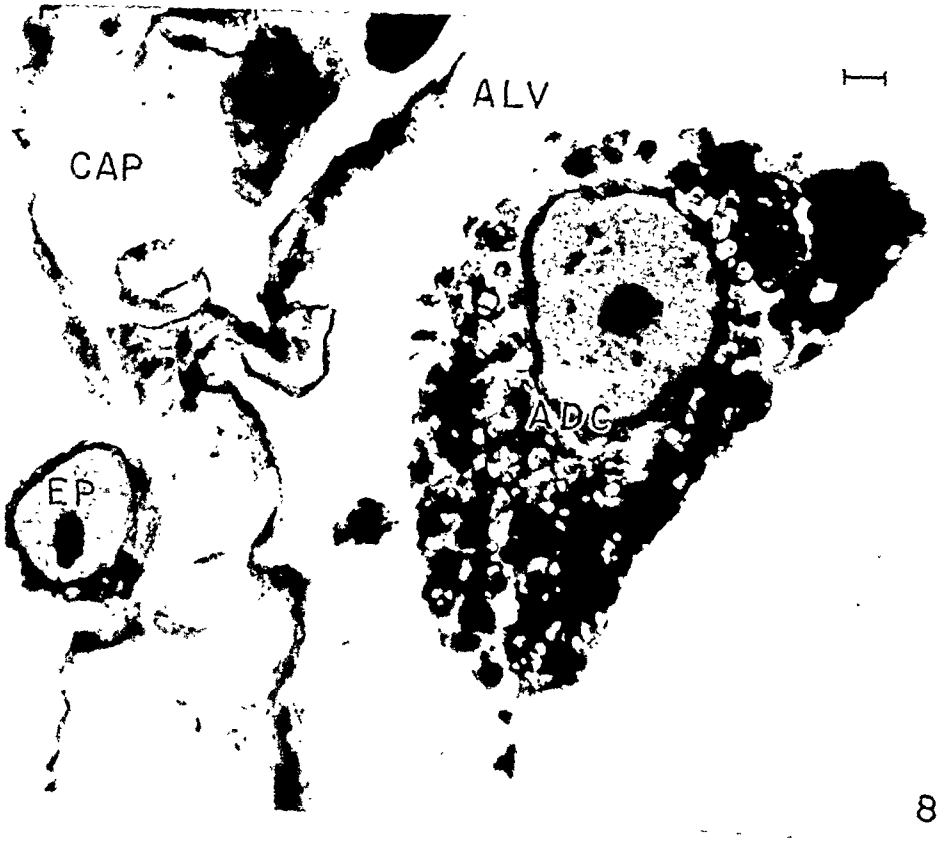
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THE DEVELOPMENT OF THE CEREBELLUM OF THE PIG

O. LARSELL

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TWENTY-THREE FIGURES

The writer recently has described in some detail the developing and the adult cerebellum of the white rat in comparison with the organs in birds ('52). The similarity of the fundamental pattern of the vermis is striking. Because the rat's cerebellum represents a rather generalized type of the organ, as it occurs in mammals, it is desirable for comparison, to present the principal results of a study of a more specialized type. The pig was selected because its cerebellum shows many variations from the generalized pattern, and because embryos are easily available.

In order to make feasible the study of closely graded stages and of many specimens of each stage, the method of dissection, rather than of reconstruction of models, was employed. Embryos fixed in 10% formalin or in Bouin's fluid were placed in 70% alcohol for several days and then were transferred to 80% alcohol until needed. After lightly surface-staining the cerebellar region with carmine the pia mater was carefully removed under the dissecting microscope, the exposed brain surface then being lightly stained. The developing fissures and other features were clearly revealed by this procedure. Enlarged photographic prints of individual cerebella then were compared with the corresponding original specimen, under the dissecting microscope at magnifications of 6 to 24 diameters, and the photographs were retouched, as needed, to bring out more strongly the fissures and other features. Figures 13–20

reproduce such enlarged and retouched photographs. Drawings of earlier stages similarly prepared but more difficult to photograph were made by tracing outlines and fissures with the aid of the camera lucida and shading as needed.

Midsagittal sections were made with a safety razor blade and likewise surface-stained with carmine. The two halves of each cerebellum so sectioned thus were available for comparison with the lobules and fissures shown in the sagittal sections. Serial sections, stained by the usual methods, were available for comparison with many of the stages dissected. For some of the later stages, in which the vermian lobules and fissures are complex, the midsagittal sections of these series rather than the dissected specimens were used for illustrations, but only after close comparison with the dissections. Shrinkage and distortion, in most cases, were greater in the paraffine embedded and serially sectioned cerebella than in those prepared by dissection.

This investigation was begun at the University of Oregon Medical School, where I was assisted in its earlier phases by Dr. Arthur G. Denker and Dr. Leonard B. Hanson.

DESCRIPTIVE

The pig embryo of 12 mm length shows a commissure in the roof of the 4th ventricle immediately caudal to the midbrain but situated in the metencephalic division of the embryonic brain. At this stage fusion of the bilateral halves of the cerebellum has begun, rostrally, along this commissure. On the external surface of the cerebellum a furrow, extending from the lateral wall of the medulla oblongata to the midplane of the fused region of the cerebellum, divides the organ into two bilaterally represented portions, the beginnings of the flocculonodular lobe and the corpus cerebelli (fig. 1). The furrow is the posterolateral fissure which is the first to appear in the mammalian embryo as in other vertebrates. Bradley ('03) who designated it fissure IV, recognized it as the first

to appear in the pig and the rabbit, and also that it is associated with the rhombic lip.

The flocculonodular lobe is continuous with the vestibular and the cochlear regions of the medulla oblongata. Dorsal to the entrance of the roots of the VIIIth nerve the rhombic lip expands into a dorsoventrally broad area. This expanded part was designated the pontobulbar body in bat and opossum embryos described by the author ('35, '36) but it is not at all clear that it corresponds to the pontobulbar body as defined by Essick ('07). It does, however, correspond to the external or inferior fold of the auricle of birds and lower vertebrates. Golgi and Cajal preparations of the corresponding fold of fetal and young rats show that the ascending rami of cochlear nerve fibers terminate in it, so that it represents the tuberculum acusticum. As the rhombic lip turns upward and medially from this inferior fold it projects laterally to a slight degree at the 12 mm stage, giving the first indication of the flocculus. A shallow groove delimits it, on each side, from the corpus cerebelli. This groove, corresponding to the paraauricular sulcus of lower vertebrates, becomes continuous around the lateral and posterior borders of the cerebellum with the corresponding furrow of the opposite side, the resulting furrow constituting the posterolateral fissure. In dissected specimens, lightly stained with carmine, the boundary between the germinal zone and the flocculonodular lobe can be seen by reason of the darker coloring of the germinal zone, owing to its content of closely arranged cells. In early stages the germinal zone is small, but it expands in subsequent development of the embryo (figs. 3–7). The choroid tela attaches to its caudal border. These features are readily recognizable in sagittal sections of embryos stained by the usual histologic methods.

Flocculonodular lobe

While the flocculus is recognizable in the 12 mm embryo the nodulus is not distinguishable from the tenial margin until approximately the 50 mm stage, when a cortical swelling be-

gins to form immediately behind and beneath the posterolateral fissure. The germinative zone in the tenial margin enlarges, its closely packed cells giving it a very different aspect from the looser structure of the incipient nodulus. When first recognizable the latter is a bilateral structure which, however, soon fuses across the midplane into a single vermian mass, as in the human embryo ('47) and in birds ('48).

In the 54 mm pig fetus the flocculus forms an expanded lateral swelling of the flocculonodular lobe (fig. 3) from which tapers medially a continuation with the nodulus. In the 62 mm fetus the flocculonodular lobe, posterolateral fissure, and acoustic tubercle are prominent (fig. 4). With caudal expansion of the corpus cerebelli, in subsequent stages, the nodulus is crowded ventrally and rostrally into the 4th ventricle, and becomes hidden from view. The connecting band between nodulus and flocculus is gradually attenuated into a slender peduncle which lies parallel to the peduncle of the ventral paraflocculus.

Lobule X, the nodulus, together with the germinative zone, forms a triangular lobule, as seen in sagittal section, the downward directed apex of which is continuous with the posterior medullary velum. The posterior surface curves forward into the posterolateral fissure, and only this surface is underlaid by early cortex. By the 90 mm stage (fig. 7 A) the posterior surface has expanded so that it bulges caudally, deepening the posterolateral fissure above it and forming a shallow furrow below, approximately at the dorsal border of the germinative zone. This appears to be the furrow called sulcus taeniae by Hochstetter ('29) in the human embryonic cerebellum, the posterolateral fissure being called the fissura anonyma by this investigator. Continued expansion of the posterior portion of the nodulus forces its ventricular surface forward, the entire mass of the nodulus gradually encroaching on the ventricular space that in earlier stages forms a large-based triangle in sagittal section. The germinative zone and the attached posterior medullary velum take off from its anterior surface, the germinative zone tapering caudally to a

tenia. By the 118 mm stage two furrows divide the posterior surface into three folia. A furrow on the ventral surface appears in fetuses of about 160 mm, dividing this surface into two folia (fig. 9). In early stages the lateral connection of nodulus with flocculus is massive, but gradually a more and more attenuated floccular stalk is formed, to the posterior margin of which the posterior medullary velum is attached. The floccular stalk is delimited from the stalk of the ventral paraflocculus by a narrow furrow, barely discernible in the later stages of the pig's cerebellum, but its developmental history and its relations clearly show this to be the posterolateral fissure. The flocculus continues laterally and rostrally beneath the ventral paraflocculus to the rostral border of the cerebellum. By the 170 mm stage of the fetus it shows two to three folia.

Corpus cerebelli

The corpus cerebelli of the 12 mm pig embryo forms an upward expansion of the remainder of the metencephalic portion of the alar plate. The two sides have begun to fuse rostrally, as already stated, but at this stage the midplane region, although including cells in addition to commissural fibers, is relatively thin. Lateroventrally the walls thicken and serial sections show that they include a relatively thick mantle layer of loosely arranged cells outside the germinal layer lining the ventricle. The ventricular surface of the cerebellum shows no boundaries between corpus cerebelli and flocculonodular lobe. It forms a rounded dome, continuous caudally with the choroid tela, but the margin of the cerebellum is clearly differentiated from the thin tela. The rostral part of the corpus cerebelli has a thicker wall, on either side of the midplane, than the more lateral and caudal portions. As viewed from the lateral aspect the cerebellar plate arches upward from the rostral end of the medulla oblongata. A strand of fibers from the trigeminal root is visible on the surface of the lower portion of the corpus cerebelli (fig. 1).

By the 20 mm stage the corpus cerebelli has thickened greatly and the medial fusion above the ventricle has extended caudally (fig. 2). There still remains, however, a deep rostrally directed notch bordered by the dorsomedial continuation of the flocculonodular lobe. The posterolateral fissure, tuberculum acusticum, and cerebellar tract of the trigeminus

ABBREVIATIONS

I, vermian lobule I
II, vermian lobule II
III, vermian lobule III
IIIA, ventral sublobule of lobule III
IIIB, dorsal sublobule of lobule III
IV, vermian lobule IV, the ventral lobule of culmen
V, vermian lobule V, the dorsal lobule of culmen
VI, vermian lobule VI, the declive
VII, vermian lobule VII, the folium and tuber vermis lobule
VIIA, anterior sublobule of lobule VII
VIIB, posterior sublobule of lobule VII, the posterior tuber vermis
VIII, vermian lobule VIII, the pyramis
IX, vermian lobule IX, the uvula
X, vermian lobule X, the nodulus
a and other small letters of alphabet indicate folia of respective vermian lobules labelled by Roman numbers
br. po., brachium pontis
bu. po. ext., bulbopontine extension
c. cb., corpus cerebelli
cr. I, crus I
cr. II, crus II
f. apm., ansoparamedian fissure
f. ic. 1, intracentral fissure 1
f. ic. 2, intracentral fissure 2
f. icul. 1, intraculminate fissure 1
f. icul. 2, intraculminate fissure 2
f. in. cr., intercrural fissure
f. pc., preculminate fissure
f. p. l., posterolateral fissure
f. ppd., prepyramidal fissure
f. ppd. b., bottom of prepyramidal fissure
f. ppd., pm., posterior margin of prepyramidal fissure
f. pr., fissura prima
f. prc., precentral fissure
f. p. s., posterior superior fissure
f. sec., fissura secunda
fl., flocculus
gn. V, semilunar ganglion
g. z., germinative zone
isth., isthmus
l. ans., ansiform lobule
l. ant., anterior lobe of corpus cerebelli
l. pmd., paramedian lobule
l. sim., lobulus simplex
med. obl., medulla oblongata
mes., midbrain
nod., nodulus
N. V, root of trigeminal nerve
N. VII, root of facial nerve
N. VIII, root of acoustic nerve
pfl. ac., accessory paraflocculus
pfl. d., dorsal paraflocculus
pfl. v., ventral paraflocculus
pl. ch., choroid plexus
po., pons
p. pfl. v., peduncle of ventral paraflocculus
de. 1, declival fissure 1
de. 2, declival fissure 2
s. uv. 1, uvular sulcus 1
tu. ac., tuberculum acusticum
v. m. a., anterior medullary velum
v. m. p., posterior medullary velum
v. 4, fourth ventricle
x, copular sulcus

are well shown at this stage. Subsequent development consists, for a considerable period, chiefly of growth in volume of the corpus cerebelli and completion of the arch above the 4th ventricle, the midplane region becoming massive. The corpus cerebelli broadens so as to project laterally well beyond the margins of the medulla oblongata, the lateral portions arching downward on each side. As seen in lateral view at the 54

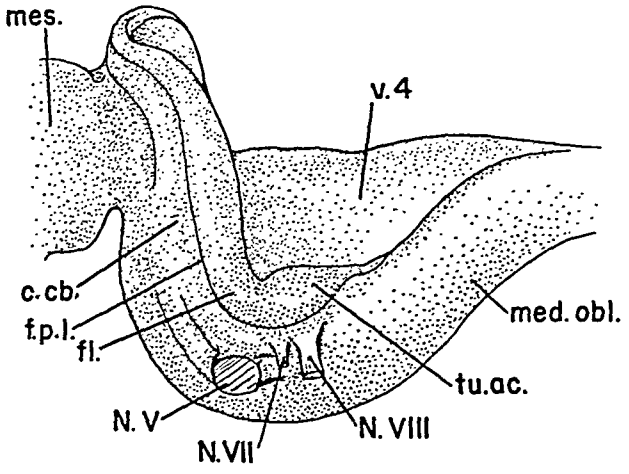


Fig. 1 Lateral view of cerebellum of 12 mm pig embryo. Camera lucida, × 16.

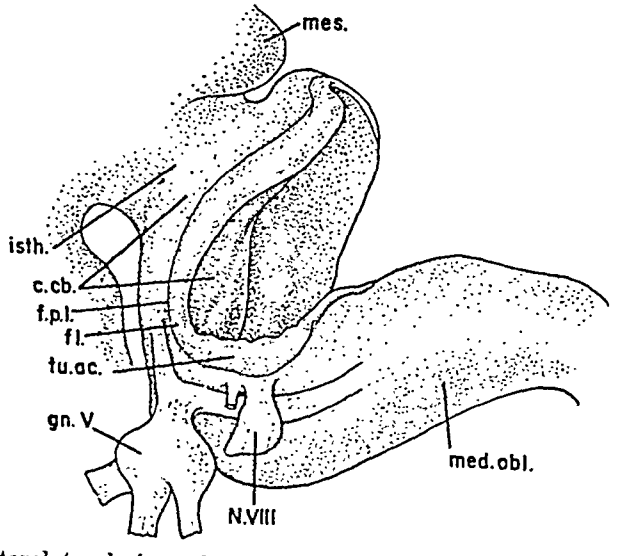


Fig. 2 Posterolateral view of cerebellum of 20 mm pig embryo. Camera lucida, × 12.

mm stage (fig. 3) the corpus cerebelli has a pear-shaped contour, bordered caudally by the flocculonodular lobe, the flocular portion of the latter continuing into the tuberculum acusticum. The increased volume of the corpus cerebelli results in upward arching, giving a rounded contour to its external surface. The ventricular surface forms a fastigial peak in the midplane but is more rounded beneath the lateral portions of the corpus cerebelli. In specimens surface-stained

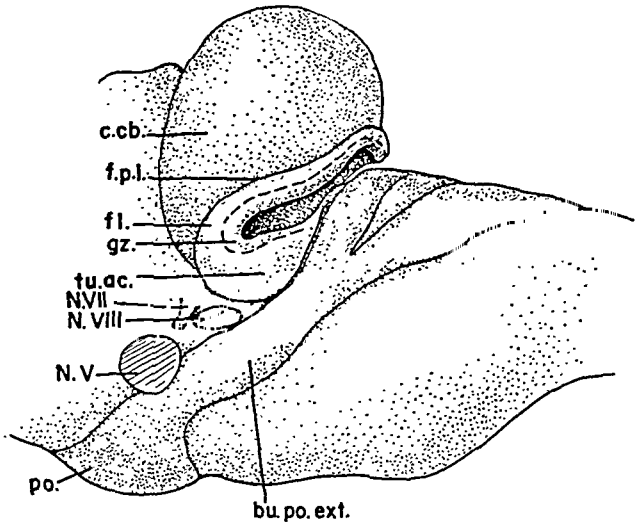


Fig. 3 Lateral view of cerebellum, pons and bulbopontine extension of 54 mm pig embryo. Camera lucida, × 12.

with carmine the margin of the incipient cortex stands in contrast to the lighter staining dorsolateral surface of the medulla oblongata. There is as yet no sign of fissure formation in the corpus cerebelli. By the 54 mm stage the bulbopontine extension, representing migratory cells from the dorsal column of the medulla oblongata, has appeared (fig. 3). The pons, which is the terminus of the migrating cells, has begun to develop, forming a small swelling on the ventral surface of the rostral part of the medulla oblongata.

At the 62 mm stage the fissura prima has appeared as a short furrow about midway between the anterior and the posterior margins of the corpus cerebelli (fig. 5). It crosses the midplane, extending a short distance on each side. The in-

cipient cortex immediately beneath the fissure is somewhat thickened and folds downward. The flocculus has expanded laterally and rostrally and a constriction has appeared between the flocculus and the tuberculum acusticum. The latter

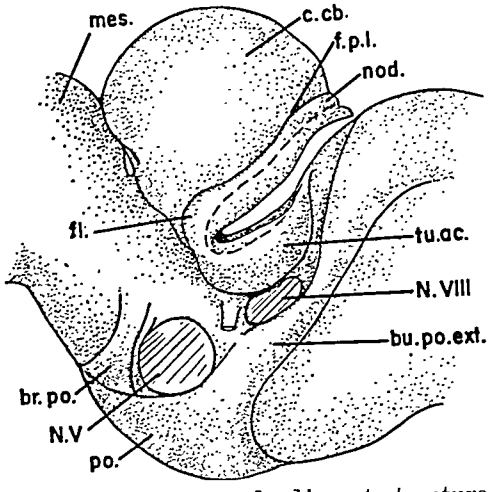


Fig. 4 Lateral view of cerebellum and adjacent structures of 62 mm pig fetus. Camera lucida, × 12.

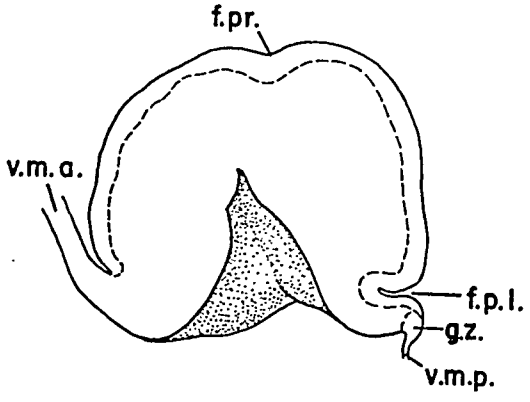


Fig. 5 Sagittal section of cerebellum of 62 mm pig fetus. Camera lucida, × 24.

is a broad, flattened shield, tapering caudomedially into the tenia (fig. 4). The bulbopontine extension and the pons are more prominent than in the 54 mm stage. A strand of fibers extending from the pons toward the cerebellum, but not yet reaching the latter, is shown. This is an early stage of the brachium pontis. The lateroventral margin of the cerebellar

cortex extends from the ventral margin of the paraflocculus to the anterior medullary velum.

At the 66 mm stage the pons has enlarged, but the bulbopontine extension appears relatively smaller than in previous stages. The brachium pontis band has reached the cerebellum, which it enters beneath the ventral margin of the incipient cortex. The corpus cerebelli appears nearly smooth save for the fissura prima. In sagittal section, however, the posterior lobe shows the first indication of the fissura secunda, the fissura prepyramidalis and the ansoparamedian fissure of Jansen

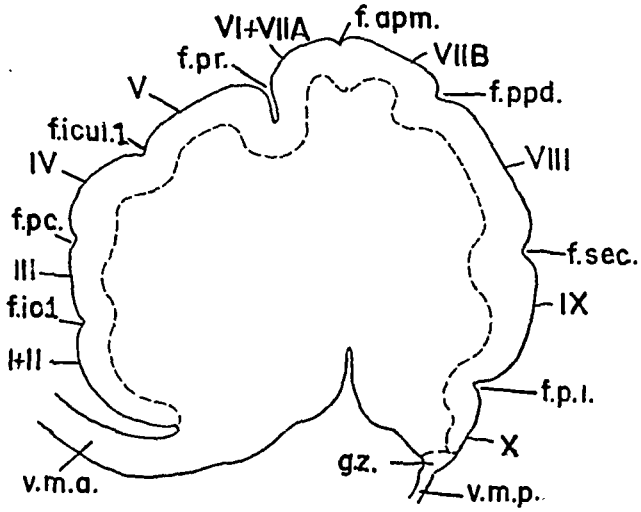


Fig. 6 Sagittal section of cerebellum of 66 mm pig fetus. Camera lucida, × 24.

(fig. 6). It is difficult to determine whether the fissura secunda appears before the prepyramidal fissure, but the various stages examined so suggest. Both precede the ansoparamedian fissure. In the rat the latter is small or absent in the vermis and the posterior superior fissure follows the prepyramidal in sequence of appearance. The declival region of the pig, however, is much more retarded in its development than is the part of the vermis between the prepyramidal and ansoparamedian fissures. Sagittal sections of the anterior lobe show three shallow furrows, with thickening of the underlying cortex. These are the preculminate, intraculminate 1, and intracentral 1 fissures, the latter delimiting incipient lobule II from

lobule III. In recent papers on the rat, cat and monkey cerebellum ('52, '53) the corresponding fissure is shown as clearly delimiting lobule II from lobule III. Subsequent studies of the human cerebellum, embryonic and adult, and of that of the anthropoid apes now in preparation for publication, clearly show that the precentral or prelingual fissure of human anatomy usually includes the fissure under consideration.

Bolk ('06), Riley ('29), and others have based the vermian lobules on the medullary rays leading to subdivisions of the cortex in the adult mammalian cerebellum. The cortical subdivisions, however, appear long before rays can be recognized. The fibers leading to and from the cortex remain diffuse for some time after the fissures appear. Only as the lobules become elongated and constricted basally with continual expansion of the cortex and formation of secondary and tertiary folds, do the fibers become aggregated into bands. In sections these appear as radiating branches of the central medullary mass. While the pattern of these rays is similar in each species, it nevertheless varies. There is a much greater variation from species to species; this has led to much confusion in the interpretation of the vermian lobules. These lobules vary in size with their functional importance in different species, as is true of other parts of the nervous system. The fiber bands leading to them become arranged in a pattern that varies in different species according to the growth stresses imposed upon them by differences in the amount of cortical expansion. As a result several lobular divisions of the cortex may be connected with the main medullary mass by a single basal extension of the latter, the branches to the individual lobules being given off at some distance from the medullary body.

In the 90 mm fetus (fig. 7 A) all of the vermian lobules are delimited by shallow furrows across the midplane, save that lobules I and II still form a common cortical fold. The fissura prima has become prominent, the corpus cerebelli now being clearly divided into anterior and posterior lobes.

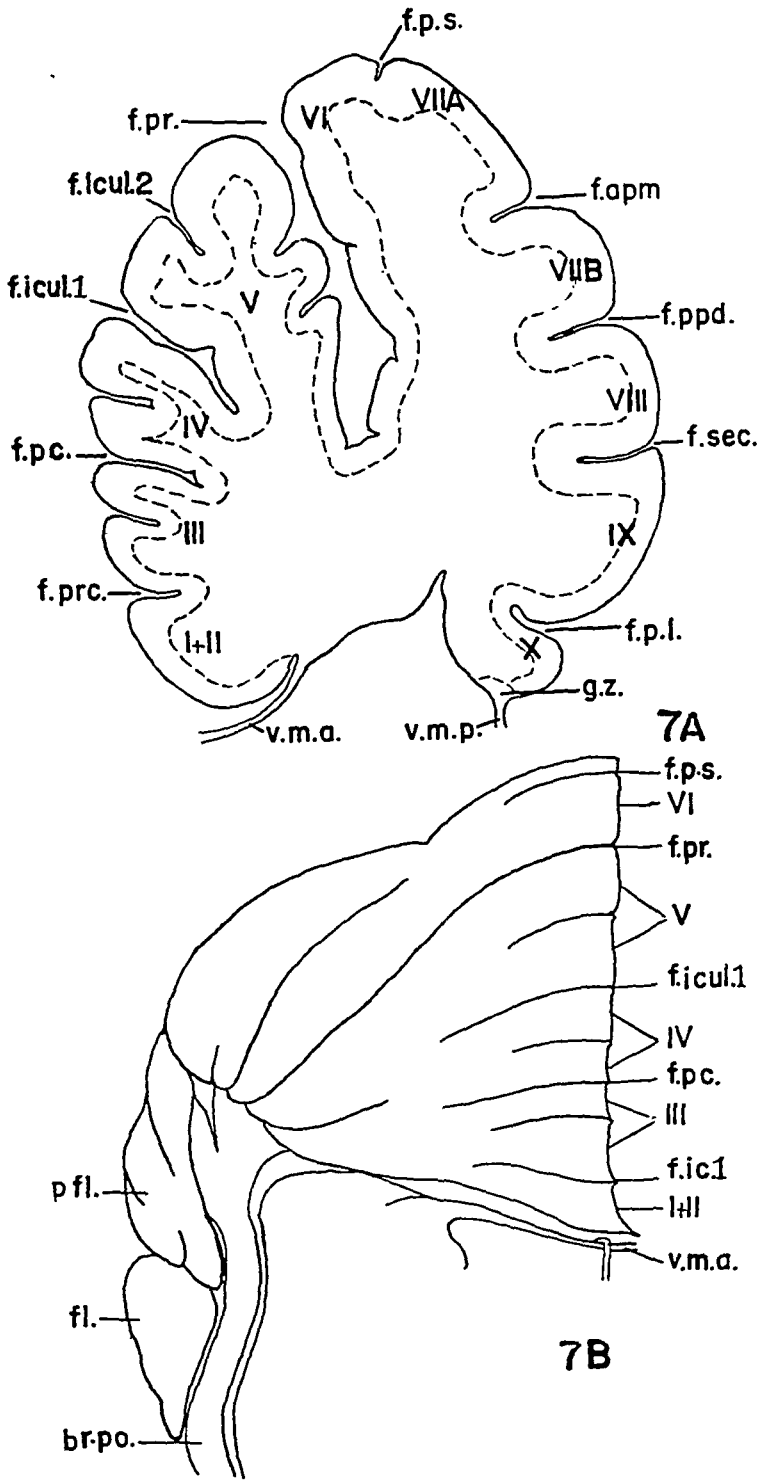


Fig. 7 Cerebellum of 90 mm pig fetus. Camera lucida, × 16. A. Sagittal section by dissection. B. Anterior view of right half.

Anterior lobe of corpus cerebelli

Lobules I + II, III, IV and V are well marked off medially by their delimiting fissures at 90 mm (fig. 7) but only the fissura prima extends laterally to the hemispheral margin. The preculminate fissure has a vermian and a hemispheral segment at this stage in the specimen illustrated in figure 7 B. In other

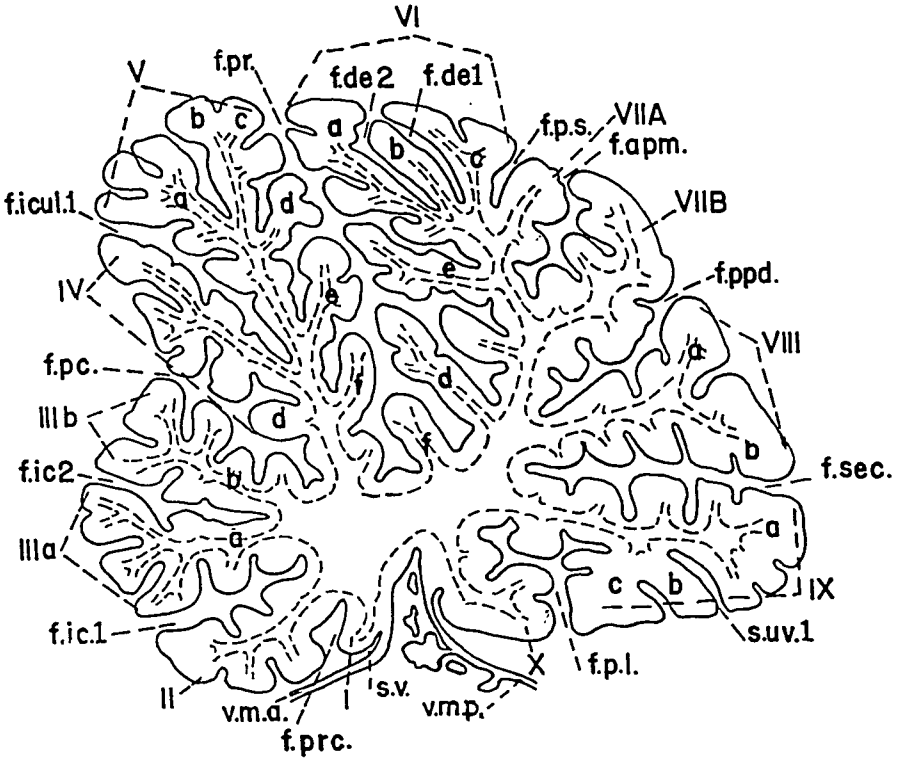


Fig. 8 Midsagittal section of cerebellum from sagittal series of 150 mm pig embryo. Projection apparatus, × 14.

fetuses of about the same size it is continuous, and in still others the hemispheral segment appears directed toward intraculminate fissure 1. There is considerable variation in the secondary foliation of lobules III and IV at this and subsequent stages, lobule III sometimes having three folia instead of the two illustrated in figure 7 A. In such cases lobule IV may have no secondary cortical folds in the 90 mm fetal stage. In the cerebellum of the 150 mm fetus, illustrated in figure 8,

the two folia of lobule III have developed into laminae IIIa and IIIb.

As the cortex burgeons outward the fissures deepen and also extend laterally toward the cerebellar margin. The latter becomes hidden from surface view by overlapping growth. In a fetus of 173 mm the precentral, preculminate, and intraculminate 1 fissures, i.e., those delimiting lobules III, IV and V, are continuous from the margin of one side to the other (fig.

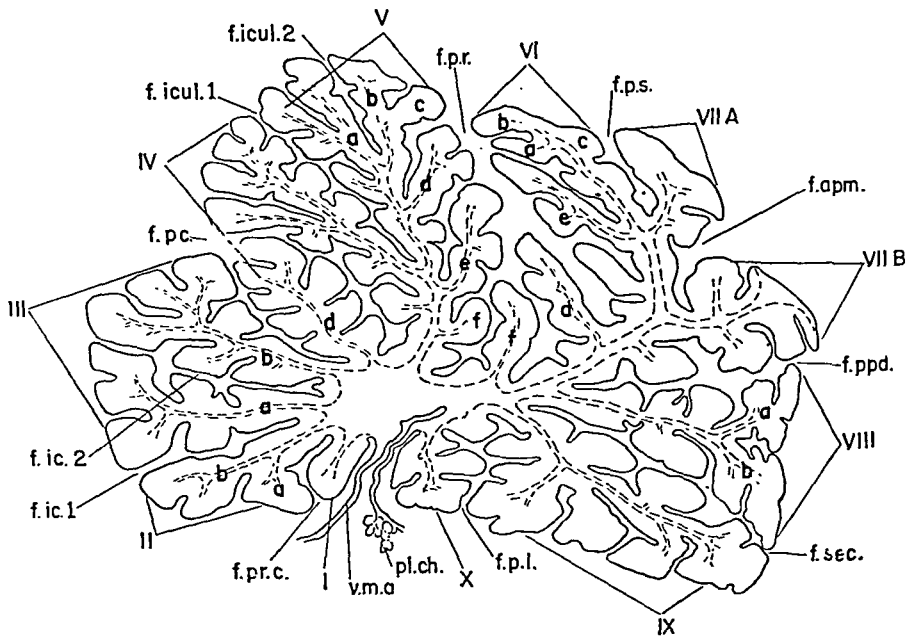


Fig. 9 Midsagittal section of cerebellum from sagittal series of brain of 170 mm pig fetus. Projection apparatus, × 10½.

10), as is the fissure between lobule I and lobule II. The latter lobules have become differentiated from the common ventrorostral cortical fold by the 150 mm stage (fig. 8). The intralobular fissures do not reach the hemispheral margin, most of them being represented only in the vermis (fig. 10). This continues true in the three-month-old pig, save for intraculminate fissure 2, which reaches the lateral margin of the hemisphere.

At the 173 mm stage the vermian lobules of the anterior lobe have assumed their adult pattern. Lobules I and II may be regarded as having no lateral representation, although lobule

II at this stage shows a narrow lateral fold suggestive of the vinculum. In the three-month-old pig this fold has been carried ventrally with the keel-like main portion of the lobule.

Lobule III, at the 150 mm and 170 mm stages, shows well differentiated laminae IIIa and IIIb already noted. Lobule IV in the 150 mm specimen illustrated in figure 8 has a medullary ray in common with lobule V. Lamina IVd is small in this specimen. In the later stage (fig. 9) what is regarded as the corresponding lamina is much larger, but it is attached directly

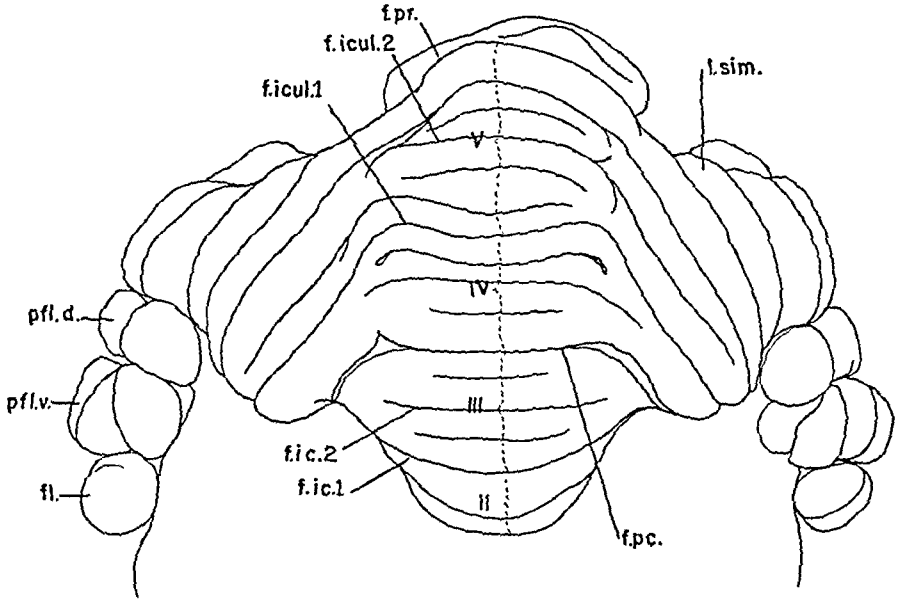


Fig. 10 Anterior view of *cerebellum* of 173 mm pig fetus. Camera lucida, × 5.

to the central medullary mass, appearing more closely related to lobule III than to lobule IV. In the three-month-old pig lobule III comprises two large divisions that may be regarded as sublobules (figs. 21, 23). The stages cited illustrate the great variation of pattern found in lobules III and IV, which are probably functionally related.

Lobule III extends somewhat beyond the lateral border of lobule II (fig. 10). Lobules IV and V are well represented on the anterior surface of the hemisphere by broad folia that correspond, collectively, to the anterior semilunar lobule of

the human cerebellum. They are larger in the three-month-old pig and spread farther laterally, but their pattern is unchanged. In lobule V laminae *a*, *b* + *c*, *d*, *e* and *f*, corresponding to those similarly designated in the monkey and the cat ('53), are recognizable at the 150 mm stage. In the young pig they have enlarged to such an extent that lobule V, in sagittal section, is the most extensive of the vermian lobules (fig. 23).

Posterior lobe of corpus cerebelli

The posterior superior fissure is not represented in the vermis until the 90 mm stage (figs. 7 and 14). The rostral part of the rapidly expanding hemisphere soon begins to fold, forming a groove which is the lateral segment of this fissure. The two segments conjoin by about the 95 mm stage, although there is much variation in different specimens. In the 125 mm specimen illustrated in figure 17, lobule VI and its lateral continuation are completely delimited from sublobule VIIA and the ansiform lobule, which is the hemispheral continuation of the latter. The band of cortex rostral to the posterior superior fissure corresponds to the lobulus simplex of Bolk. The vermian part is the declive of human anatomy, while the lateral part corresponds to the posterior semilunar lobule of the human cerebellum. Before the posterior superior fissure reaches the hemisphere, laterally, a furrow has appeared rostral to it, in the declive, representing declival sulcus 1. In the pig embryo the rostral portion of the declive is tilted forward into the outer part of the fissura prima. A shallow furrow, declival sulcus 2, is visible in sagittal section of the cerebellum at about the 118 mm stage of the fetus. The declival portion of lobule VI thus is divided into folia VIa, VIb and VIc, as in the rat (figs. 8, 9). In the 150 mm and later stages laminae VId, VIe and VIf are distinct in the posterior wall of the fissura prima; they resemble the corresponding cortical folds in cat and monkey.

Following the differentiation of lobules VI and VII from each other by the posterior superior fissure, lobule VII and its

laterally connected lobules begin to grow rapidly. In the rat lobule VII remains rather simple, frequently showing no sign, medially, of the ansoparamedian fissure; when present this fissure is but a shallow furrow in the vermian region. In the pig, however, lobule VII is broad, rostrocaudally, and already is divided into two parts by the ansoparamedian fissure in the 90 mm fetus (figs. 7 A, 14). These parts represent the beginning of sublobules VIIA and VIIB. There is as yet no suggestion of lateral displacement of any segment of the vermis. Save for the enlargement and subdivision of lobule VII, minor variations in the other lobules, and the retarded differentiation

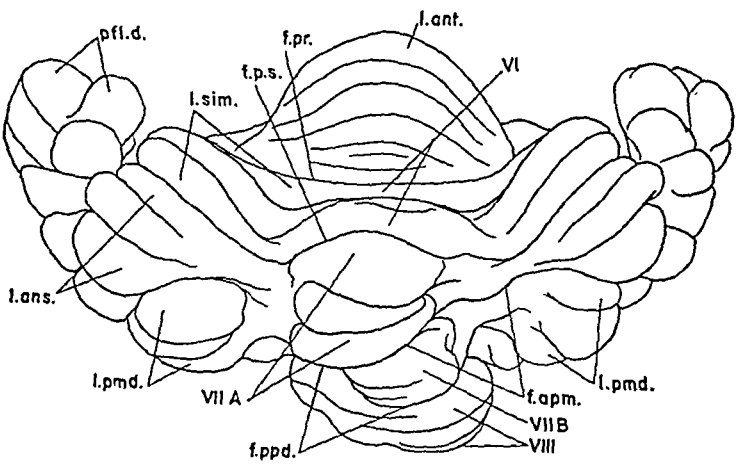


Fig. 11 Dorsal view of cerebellum of 143 mm pig fetus. Camera lucida, × 5.

from each other of lobules I and II, the vermis of the pig fetus at this stage of development is essentially similar to that of the rat 4 to 5 days after birth (cf. '52, figs. 18, 25, 29). In the 110 mm pig fetus the vermian part of the ansoparamedian fissure is deep and also has joined its hemispheral segments. Sublobule VIIA now is clearly continuous laterally with the ansiform lobule, and VIIB with the paramedian lobule (fig. 16).

Sublobule VIIB becomes divided by a shallow sulcus into two surface folia by the 140 mm stage (fig. 18). Shallow sulci, extending into its posterior surface from the prepyramidal fissure, divide this surface into three main folia. At about the 140 mm stage occurs the first indication of displacement (figs.

11, 18) of this sublobule toward the right hand side of the cerebellum as a rule, but sometimes to the left. In a fetus of 150 mm this displacement is well advanced. It is accompanied by displacement of sublobule VIIA toward the opposite side. The ansoparamedian fissure between the two has become a broad cleft on the left side of the vermis by the 150 mm stage (fig. 19) extending beneath the caudal folia of VIIA, which form a plate above the fissure at this stage. The floor of the paramedian fissure shows no indication of folia at the 150 mm stage, but there is a thin layer of cortex extending over most of it, between the vermian and the lateral extensions of sublobules VIIA and VIIB. In a fetus of 163 mm, however, narrow folia connect VIIA with crus I and crus II, but VIIB is still connected with the paramedian lobule, laterally, only by a thin sheet of cortical substance, as judged from the staining reaction with carmine. Between this sheet and the folium connecting sublobule VIIA with crus II a narrow zone, which is unstained, represents the ansoparamedian fissure as it crosses the hollow of the paramedian fissure.

In further growth of the cerebellum the superficial portions of sublobules VIIA and VIIB are displaced farther to the left and the right, respectively. Their deep portions become flattened stalks of attachment to the common cerebellar mass which is served by a single primary medullary ray that also serves lobule VI, as well as the foliated cortex on the posterior wall of the deep portion of the fissure prima (figs. 8, 9, 23). Beginning at about the 110 mm stage of the fetus, sublobules VIIA and VIIB appear to be more distinct from each other than VIIA is from lobule VI. Their developmental history, when compared with that in the rat and other mammals, shows that they are derived from a common primary vermian segment.

Lobule VIII can be recognized in surface view by the 80 mm stage. The prepyramidal fissure, which delimits it rostrally, is present in the vermian region. In sagittal section of a 66 mm fetus this fissure is a shallow groove (fig. 6). By the 90 mm stage it has deepened considerably (fig. 7 A) and it reaches

almost to the median portion of the paramedian lobule, whose outswelling has extended farther medially, as also has the paraflocculer fissure caudal to the paramedian lobule. A narrow zone of cortex, however, crosses obliquely from the pyramis to the paramedian lobule between the ends of the two fissures (fig. 14). In the 140 mm fetus this connection has become reduced to a narrow strip, shown on the left side in figure 18, but on the right of this specimen it was hidden in the depths of the prepyramidal fissure which extended to the paraflocculer fissure. A connection on the right side is shown in figure 20 (170 mm fetus). This conection between pyramis and paramedian lobule will be considered further in the description of the later development of the paramedian lobule.

The lateral displacement of sublobule VIIB draws with it the pyramis. At first only the upper folia are involved, but gradually the lower folia of the pyramis and also the upper folia of lobule IX, the uvula, are drawn toward the right hand side. The result is the sinuous posterior part of the vermis of the pig. A similar form is found in many species. It is more S-shaped in the cat, horse, ox and many other carnivores and ungulates, and less so in the sheep and a number of other animals, as is shown also in the figures of Bolk ('06) and Riley ('29). It is important to recognize that this S form of the vermis caudal to the posterior superior fissure in the pig results from the great enlargement of lobulus VII, and that the first or more anterior segment is formed by sublobule VIIA, the folium-tuber lobule; the second segment by sublobule VIIB, the posterior tuber; the third segment represents lobule VIII, the pyramis; and the 4th or inferior segment is formed by lobule IX, the uvula. Lobules VIII and IX are only slightly displaced to the right in the pig fetus in contrast to the condition in the cat, in which VIIB and VIII show the greatest displacement. In the three-month-old pig lobule IX has returned to a medial position (fig. 22).

In sagittal section lobule VIII is relatively narrow, dorsoventrally, in fetal stages subsequent to the onset of the lateral displacement of the adjacent parts as described. This is true

also in the adult pig as compared with adult stages in many other species. At the 150 mm stage of the pig fetus the lobule is divided into two superficial folia, designated VIIIa and VIIIb, by a shallow furrow, the intrapyramidal sulcus. The two folia subfoliate and the sulcus deepens, but in the adult pig and in other species with a sinuous "posterior vermis" the sulcus does not attain the relative depth found in species without lateral displacement of adjacent vermian parts. The cortical surface of the pyramis, however, is not necessarily reduced in proportion to the dorsoventral measurement of the lobule. The displacement laterally toward the posterior tuber segment increases the upper and lower surfaces of the pyramis in the sinuous types of vermis as compared with the type represented in the rat, man and many other species. The principal lateral connection of lobule VII, the pyramis, is with the dorsal paraflocculus, as will be described in greater detail in another section. The bridge of cortex between the ends of the prepyramidal and paraflocculus fissures in early stages, however, also gives it a connection with the paramedian lobule (figs. 18, 20).

Lobule IX, the uvula, is delimited above by the fissura secunda, already described. By the 140 mm stage it is divided by uvular sulcus 1 and uvular sulcus 2 into folia IXa, IXb and IXc. Folia IXa and IXc already show secondary foliations. These are illustrated in the 150 mm and 170 mm fetuses (figs. 8 and 9). Laterally the lobule extends into the ventral paraflocculus.

The lobulus simplex. The vermian portion of the lobulus simplex, lobule VI, has been described. The hemispheral swelling lateral and caudal to the fissura prima is delimited, in the 80 mm fetus, by a furrow which on the basis of its subsequent development clearly is the ansoparamedian fissure. At this stage there is no indication of fissuration in this swelling but in its vermian continuation the beginning of the posterior superior fissure is apparent in sagittal section at the 90 mm stage. In the 125 mm fetus the posterior superior fissure is evident laterally as a continuation of the vermian furrow

(fig. 17). The lateral segment of this fissure divides the rostral portion of the hemisphere into an anterior region, corresponding to the lateral part of Bolk's lobulus simplex, and a posterior region, the ansiform lobule of Bolk.

In the earlier stages the lateral portion of the lobulus simplex is relatively large and it projects rostrally as an ovoid swelling, on the upper surface of which a furrow is present in the 125 mm stage. This extends laterally and medially, joining declival sulcus 1, so that 2 broad folia result. They are continuous with each other around the lateral end of the sulcus. In subsequent growth of the cerebellum the lateral portion of the lobulus simplex becomes reduced in size relative to adjacent lobules, but the declival portion becomes prominent; this part of the cerebellum in the adult pig is unusually large and projects rostrally over the fissura prima so that the external margins of this fissure are hidden from surface view.

The *ansiform lobule* is delimited from the lateral portion of the lobulus simplex by the lateral continuation of the posterior superior fissure sometime between the 110 mm and the 125 mm stages. By the latter stage three secondary folds on the left side and two on the right have appeared in the specimen illustrated in figure 17 as already noted. The furrow between the two folia on the right side of the figure, and between the more rostral and middle folia on the left side, is the intercrural sulcus delimiting crus I from crus II. It almost meets its vermian representation in lobule VIIA at this stage. At the 140 mm stage continuity has been established (fig. 18), but this is transitory on the right side of the cerebellum. The displacement to the left of sublobule VIIA, as the vermis assumes its sigmoid form, stretches and thins the cortex in the paramedian furrow to such an extent that the intercrural sulcus disappears in this region; its vermian and hemispheral segments, however, deepen with growth of the respective adjacent lobules (figs. 19 and 20). In the 150 mm fetus crus I consists of two narrow folia and crus II of a broader single folium, the latter disappearing medially in the broad paramedian furrow (fig. 19). By the 170 mm stage three folia are shown in

crus II. In the three-month-old pig the ansiform lobule continues medially into a mass of medullary substance upon which the folia of lobule VI and sublobule VIIA are superposed without continuity of cortex from the vermian to the hemispheral folia in either case. The ansoparamedian and posterior superior fissures come together as a common fissure for a short stretch but separate laterally, the latter delimiting crus I from the lobulus simplex, and the former delimiting the ansiform and paramedian lobules, as already described. In the adult pig there are 6 to 8 folia in each crus of the ansiform lobule.

Paramedian lobule. The incipient paramedian lobule is already apparent in the 80 mm pig fetus, being delimited rostrally by the lateral portion of the ansoparamedian fissure and caudally by the paraflocculus fissure. By the 110 mm stage the medial portion of the ansoparamedian fissure has joined the lateral portion on each side so that a fissure, continuous from one lateral surface of the cerebellum to the other, has resulted (fig. 16). The cortical elevations posterior to it are the incipient paramedian lobules, on each side, and sublobule VIIB, the posterior tuber, medially. The prepyramidal fissure, delimiting the latter from the pyramis posteriorly, ends before reaching the base of the triangle representing the paramedian lobule. The pyramis and the posterior tuber, accordingly, obviously are continuous with the paramedian lobule (figs. 13–17). In later stages this continuity of the pyramis is not so clear but it persists as a slender connection. The fissure between the paramedian lobule and the paraflocculus, on either side, is the paraflocculus fissure. The cortical zone between the medial portion of this furow and the lateral portion of the prepyramidal fissure is regarded as corresponding to the laterally tapering continuation of lobule VIII in the rat, which develops into the posterior portion of the paramedian lobule. In the pig, as in the rat, this lobule also consists of an anterior portion, represented medially by sublobule VIIB, and a posterior portion, related medially to lobule VIII. The early prominence of the ansoparamedian fissure in the pig appears

to be due, medially, to the large size attained by sublobule VIIA, and laterally to the prominence and dorsal position of the rostral portion of the paramedian lobule.

The vermian segment of the prepyramidal fissure does not join the paraflocculer fissure until about the 150 mm stage. Prior to this stage lobule VIII continues laterally with the paramedian lobule across the gap between the two fissures; it also continues directly into the dorsal paraflocculus (figs. 19, 20). As sublobule VIIB and lobule VIII expand and the prepyramidal fissure deepens, the vermian part of this cortical bridge to the paramedian lobule is drawn downward into the fissure. Laterally it is attenuated into a narrow band of cortex which expands distally to form the caudal folium of the paramedian lobule. As in the rat lobule VIII is thus continued laterally into the paramedian lobule, forming the posterior portion of the latter.

As late as the 95 mm stage (fig. 15) there is a similar bridge of connection between sublobule VIIB, medially, and the incipient paramedian lobule laterally. This forms a gap between the vermian and the hemispheral parts of the ansoparamedian fissure. This gap has closed in the 110 mm stage and, on one side, in the 125 mm fetus illustrated in figure 17. The ansoparamedian fissure thus becomes continuous for a time, but the portion crossing the paramedian furrow disappears as the cortex thins with lateral displacement of the hemisphere. The paramedian lobule is continuous medially with sublobule VIIB only, which by the 150 mm stage has become strongly displaced to the right (figs. 19 and 20).

As the medial and lateral portions of the ansoparamedian fissure join at the 110 mm stage sublobule VIIB and the paramedian lobule become completely delimited rostrally from VIIA and the ansiform lobule. The paramedian lobule in its earlier stages, in addition to its connection with sublobule VIIA, is broadly connected medially with lobule VIII. The paraflocculer fissure gradually extends medially as the lobule grows, constricting the cortical strip connecting with lobule VIII to a narrow band (figs. 17 and 18); sometimes, as in

figures 18 (right side) and 19, this is hidden in the depths of a furrow that as early as the 140 mm stage often connects the prepyramidal and parafloccular fissures. By a lateral continuation of this connection from the folia in the rostral and deeper portion of the prepyramidal fissure the posterior part of the paramedian lobule is formed. The anterior part of the paramedian lobule continues medially with the posterior tuber

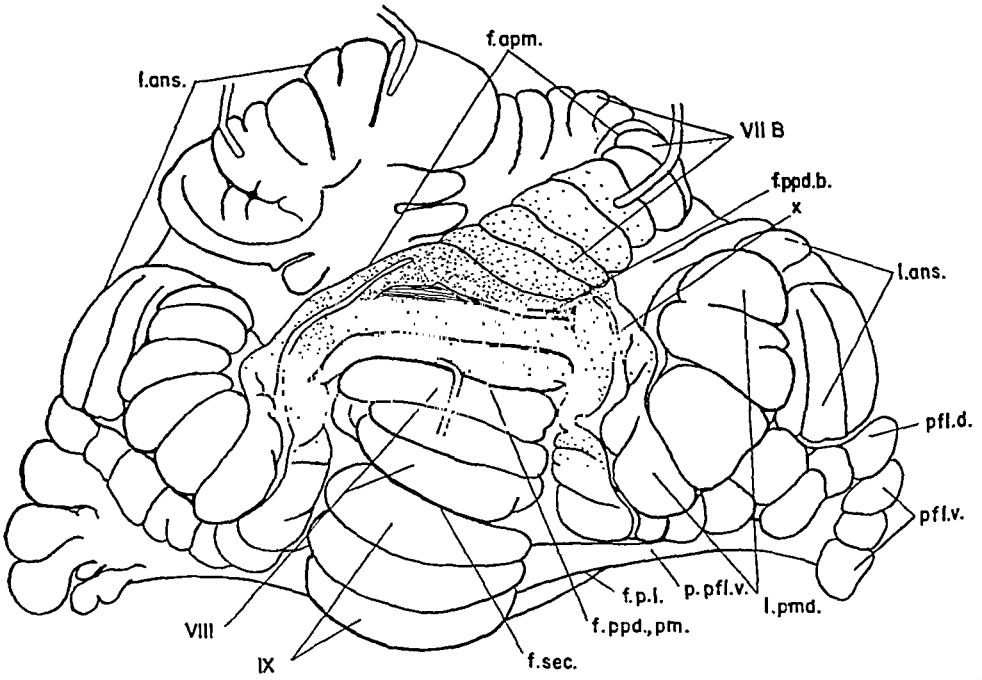


Fig. 12 Dorsal view of cerebellum of 170 mm pig fetus, in which the walls of the prepyramidal fissure are spread apart to show the deep folia and their continuity with dorsal paraflocculus and paramedian lobule, and the left ansiform lobule is retracted. × 7½.

which foliates in the meantime; by the 170 mm stage three folia are present (fig. 20). At the 170 mm stage the paramedian lobule shows 6 folia, the posterior three connected medially with lobule VIII or with folia on its anterior wall in the floor of the prepyramidal fissure. This connection is illustrated in figure 12, representing a dissection in which the prepyramidal fissure was spread open as widely as possible to reveal the lateral continuations of folia forming its anterior and posterior walls. It will be noted that the folia of the deep portion of

the posterior wall pass into a rostrocaudally directed folium on either side. This continues rostrally to the base of sublobule VIIA, and then turns sharply laterally and caudally as a stalk to the posterior portion of the paramedian lobule. There also is continuity of medullary substance from between the floor of the prepyramidal fissure and from the deep rostral portion of lobule VIII, to the paramedian lobule beneath fissure *x* which separates the stalk of the paramedian lobule from the folium that gives rise to it. Fissure *x* appears to correspond to the copular sulcus of the rat. In the specimen illustrated the portion of the stalk of the dorsal paraflocculus which is covered by cortex is attached to the caudal end of this longitudinal folium. In another specimen, not illustrated, in which the three posterior folia of sublobule VIIB were dissected away, continuity is shown from the pyramis base to the stalk of the posterior paramedian lobule and also to the stalk of the dorsal paraflocculus. Medullary substance extends between the paraflocular stalk and the pyramis below the fissure designated *x*. A thin layer of cortex also continues laterally from the pyramis to the stalk of the dorsal paraflocculus, and rostrally into cortex stretched between sublobule VIIB and the rostral portion of the paramedian lobule.

As sublobule VIIB becomes displaced farther and farther to the right, with increase in size of the cerebellum, the folia from the walls of the prepyramidal fissure are exposed at the lateral border of this fissure, which here is spread apart. The stages of this feature of development are most readily understood by reference to figures 18, 19 and 20, in which successive stages of foliation from the originally unfolded cortex of this region are illustrated. On the left side of these cerebella some of the corresponding folia may be seen by tilting the cerebellum strongly to the right, a procedure which also brings to view the folia, on the anterior wall of the prepyramidal fissure, which continue into an unfoliated sheet of cortex that passes laterally into the anterior paramedian lobule.

Paraflocculus. This lobule and its relations to adjacent parts of the cerebellum have proved difficult of analysis. Elliot

Smith ('02) who applied the name now universally used in the mammalian cerebellum, included it and the flocculus in his floccular lobe. Bolk ('06) included it in his formatio vermicularis, of which the "pars floccularis" was considered a subdivision. In 1934 I pointed out the differences in basic morphological relationships between the flocculus and the paraflocculus, and the incorrectness of including the latter under the term floccular lobe. Subsequently ('37) I introduced the term flocculonodular lobe to avoid confusion with Elliot Smith's use of the term "floccular lobe" and my definition, which would limit the lobe to flocculus and nodulus, i.e., to the primarily vestibular part of the cerebellum. While the paraflocculus has since been widely recognized as belonging to the corpus cerebelli its relations to the vermian lobules and its homologies with subdivisions of the human cerebellum have remained obscure, save for the analysis of Scholten ('46) in adult mammals. The interpretations of this author, which I regard as correct in most respects, are corroborated and further elucidated by the results of a comparative study of the development of the paraflocculus and related parts of the cerebellum in embryos of several mammalian species, including the pig; only the latter will be described in the present contribution.

It is evident from figure 17 that the paraflocculus is divided very early, as already noted, into dorsal and ventral limbs. This is brought about by the confluence of the furrow which appears on the lateral surface of the paraflocculus with the earlier formed medial segment of the fissura secunda. Stages of confluence of these segments of the fissura secunda are illustrated in figures 15–17. The completed fissure corresponds to sulcus *d* of Bradley and is regarded as the definitive fissura secunda. It entirely delimits pyramis and dorsal paraflocculus from the uvula and ventral paraflocculus; around the end of the fissure, however, the two limbs of the paraflocculus merge. These features were clearly described by Bradley ('03, '04). In the 125 mm fetus both limbs are attached to their vermian lobules (VIII and IX respectively) by a massive stalk (fig. 17).

At this stage the distal portion of the dorsal paraflocculus has begun to foliate. In the 140 mm fetus (fig. 18) foliation has extended caudomedially along both stalks, but in the ventral paraflocculus it extends only to the approximate level at which the flocculus begins to expand laterorostrally. Medialward from this point the ventral paraflocculus tapers into a narrow peduncle that again expands as it approaches lobule IX, which it joins (figs. 15 and 16). The narrow portion of the peduncle is embedded in a sheet-like lateral continuation of the posterior medullary velum. The foliated portion of the ventral paraflocculus corresponds to the uncus of the paraflocculus of the adult pig and many other mammals. The most posterior folium enlarges more than the others and is regarded as corresponding to the accessory paraflocculus of Jansen ('50). There are indications that its fibers continue medially into the inferior folia of the uvula, suggesting that it may correspond to the avian paraflocculus. The stalk of the dorsal paraflocculus is foliated nearly all the way to its attachment to the pyramis in the 170 mm fetus. In the three-month-old pig, cortex is continuous between paraflocculus and all the folia of lobule VIII with which it connects (fig. 22). As already noted, the parafloccular peduncle is also continuous with the stalk of the posterior part of the paramedian lobule.

SUMMARY

The cerebellum of the pig differs so widely from those of the rat ('52), cat and monkey ('53) that a detailed account of its development is presented to establish homologies of its fissures and lobules. The lobules are based on the divisions of the cortex by the earliest furrows, rather than on the medullary rays. The latter are condensations, as the cerebellum develops, of diffuse fibers and are not recognizable until long after the primary cortical folds are well established.

Vermian lobule I is small and differentiates relatively late in the pig. Lobule II is delimited from lobules I and III, in later stages, by deep fissures. Lobule III is divided into sublobules IIIA and IIIB. Lobule IV is prominent and distinct.

Lobule V is large and shows surface laminae Va, Vb, and Vc, corresponding to those in cat and monkey. Lobule VI is narrow in the sagittal plane owing to the small size of laminae VIa, VIb, and VIc, but its laminae in the posterior wall of the fissura prima give it an extensive cortical surface. Lobule VII is divided by a deep ansoparamedian fissure into sublobules VIIA and VIIB; these become strongly displaced, usually to the left and right respectively, producing a sinuous posterior part of the vermis. The displacement is more marked and involves sublobule VIIA much more than in the cat; lobule VIII, which is rather small, on the other hand is much less displaced in the pig. Lobule IX is large, and lobule X is small.

The hemispheral relations of the vermian lobules correspond, in general, to those of the rat, cat, and monkey. Lobule I is confined to the vermis. The anterior folium of lobule II extends into the rostral part of the paramedian furrow, as do the folia of lobule III. The latter collectively correspond to the alae lobuli centralis of man, in much reduced form. Lobule IV extends onto the anterior surface of the hemisphere as a broad folium. The lateral continuation of lobule V forms a large proportion of the anterior cerebellar surface; together with the lateral extension of lobule IV it corresponds to the anterior semilunar lobule of the human cerebellum. Lobule VI and its hemispheral continuation correspond to the lobulus simplex of Bolk; the hemispheral part is homologous with the human posterior semilunar lobule. Sublobule VIIA, the folium tuber vermis lobule, continues into the ansiform lobule; the latter is subdivided into crus I and crus II, each of which is further subdivided, but not so distinctly as in cat and monkey. Respectively they correspond to the superior and inferior semilunar lobules of man. Sublobule VIIB continues into the ansiform lobule and into the anterior part of the paramedian lobule. Folia in the deep part of the prepyramidal fissure are continuous with the posterior part of the paramedian lobule and with the dorsal paraflocculus. The latter is also continuous medially with lobule VIII, the pyramis. The ventral paraflocculus is continuous, through its peduncle, with lobule IX, the

uvula. The fissura secunda delimits dorsal and ventral paraflocculi from each other throughout their extent, but they are continuous around the rostrolateral extremities of this fissure. The flocculus is continuous by its peduncle with lobule X, the nodulus, the two parts constituting the flocculonodular lobe. This is differentiated very early.

The folia and laminae in the walls of the principal fissures, as well as those of the cerebellar surface, have patterns similar to those in the cat and monkey cerebellum in corresponding locations. They are designated by letters of the alphabet corresponding to those used in the descriptions of the cerebellum of these animals.

LITERATURE CITED

Bolk, L. 1906 Das Cerebellum der Säugetiere. Haarlem, De Erven F. Bohn; Jena. Gustav Fischer.

Bradley, O. C. 1903 On the development and homology of the mammalian fissures. Part II. Pig. J. Anat. and Physiol., n.s., *17:* 221–240.

———— 1904 The mammalian cerebellum: its lobes and fissures. Part I. J. Anat. and Physiol., n.s., *18:* 448–475.

Essick, C. R. 1907 The corpus pontobulbare — a hitherto undescribed nuclear mass in the human hind brain. Am. J. Anat., *7:* 119–130.

Hochstetter, F. 1929 Beiträge zur Entwicklungsgeschichte des menschlichen Gehirns. II. Teil, (3) Schlusslieferung. Die Entwicklung des Mittel und Rautenhirns. Wien und Leipzig, Frans Dueticke.

Jansen, Jan 1950 The morphogenesis of the cetacean cerebellum. J. Comp. Neur., *93:* 341–400.

Larsell, O. 1934 Morphogenesis and evolution of the cerebellum. Arch. Neur. and Psychiat., *31:* 373–395.

———— 1935 The development and morphology of the cerebellum in the opossum. Part I. Early development. J. Comp. Neur., *63:* 65–94.

———— 1936 Cerebellum and corpus pontobulbare of the bat (Myotis). J. Comp. Neur., *64:* 275–302.

———— 1937 The cerebellum: A review and interpretation. Arch. Neur. and Psychiat., *38:* 580–607.

———— 1947 The development of the cerebellum in man in relation to its comparative anatomy. J. Comp. Neur., *87:* 85–130.

———— 1948 The development and subdivisions of the cerebellum of birds. J. Comp. Neur., *89:* 123–190.

———— 1952 The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat. J. Comp. Neur., *97:* 281–356.

———— 1953 The cerebellum of the cat and the monkey. J. Comp. Neur., *99:* 135–199.

RILEY, H. A. 1929 The mammalian cerebellum. A comparative study of the arbor vitae and folial pattern. Res. Publ. Assn., nerve. ment. Dis., *6:* 37–192.

SCHOLTEN, J. M. 1946 De plaats van den paraflocculus in het geheel der cerebellaire correlaties. Dissertation. Amsterdam.

SMITH, G. ELLIOT 1903 The morphology of the human cerebellum. Rev. Neur. and Psychiat., *1:* 629–639.

PLATE 1

EXPLANATION OF FIGURES

13 Dorsal view of cerebellum of 80 mm pig fetus. Enlarged and retouched photograph, as explained in text.

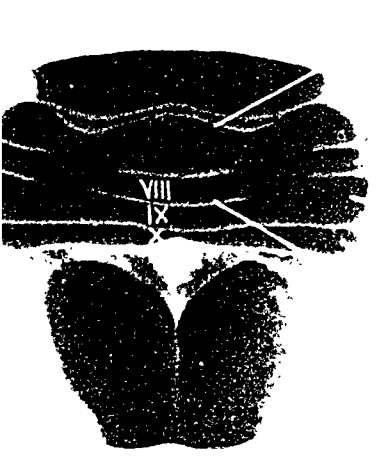
14 Dorsal view of cerebellum of 90 mm pig fetus. Enlarged and retouched photograph.

15 Dorsal view of cerebellum of 95 mm pig fetus. Enlarged and retouched photograph.

16 Dorsal view of cerebellum of 110 mm pig fetus. Enlarged and retouched photograph.

17 Dorsal view of cerebellum of 125 mm pig fetus. Enlarged and retouched photograph.

18 Dorsal view of cerebellum of 140 mm pig fetus. Enlarged and retouched photograph.



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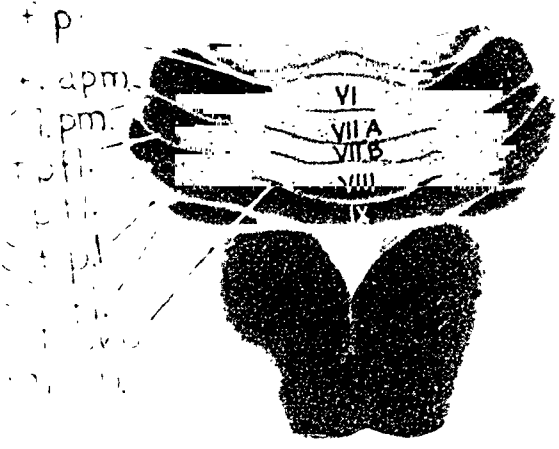

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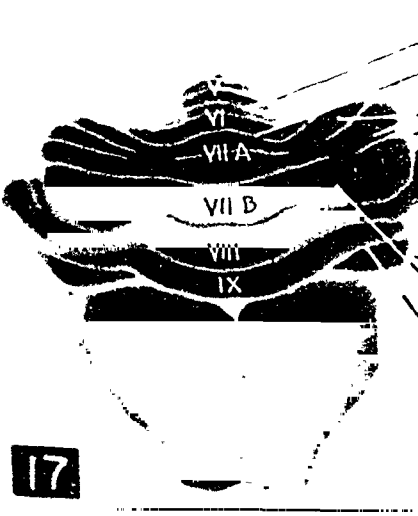

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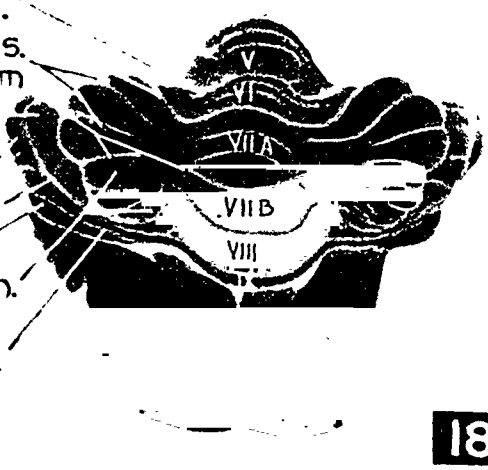

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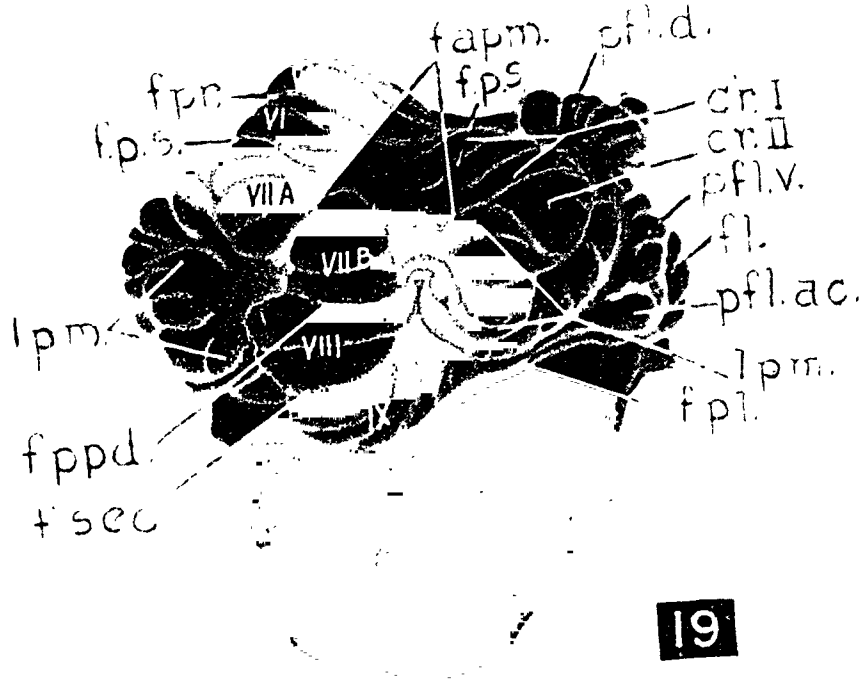
PLATE 2

EXPLANATION OF FIGURES

19 Dorsolateral view of cerebellum of 150 mm pig fetus. Enlarged and retouched photograph.

20 Dorsolateral view of cerebellum of 170 mm pig fetus. Enlarged and retouched photograph.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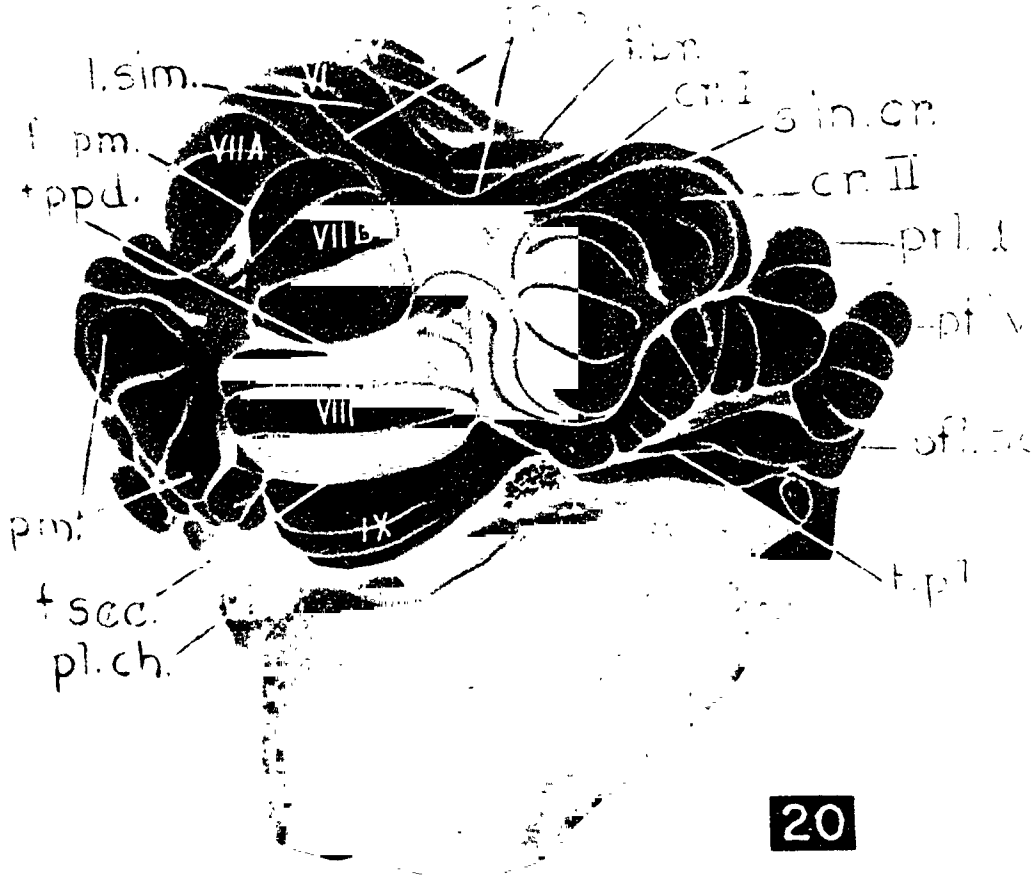
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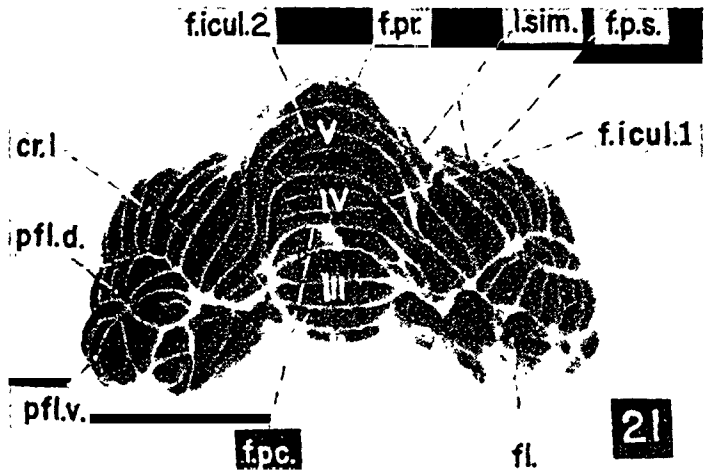
PLATE 3

EXPLANATION OF FIGURES

21 Anterior view of cerebellum of pig, age three months. Retouched photograph of dissection, surface-stained with carmine.

22 Posterior view of cerebellum of pig, age three months. In this specimen sublobule VIIB was displaced to the left. Retouched photograph of dissection, surface-stained with carmine.

23 Sagittal section of cerebellum of pig, age three months. In this specimen sublobule VIIB was displaced to the right. Retouched photograph of dissection. lightly surface-stained with carmine.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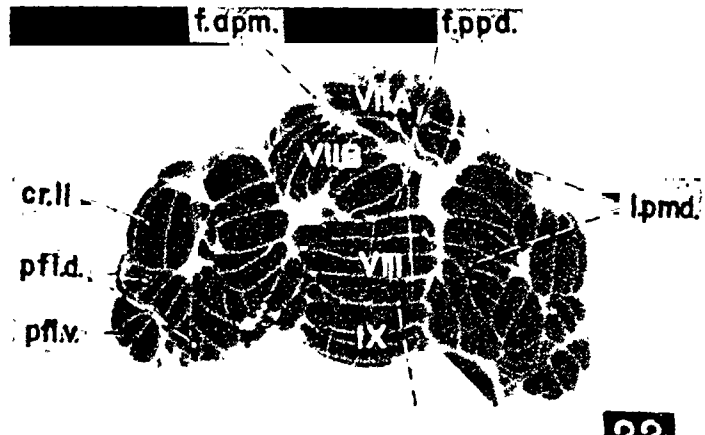
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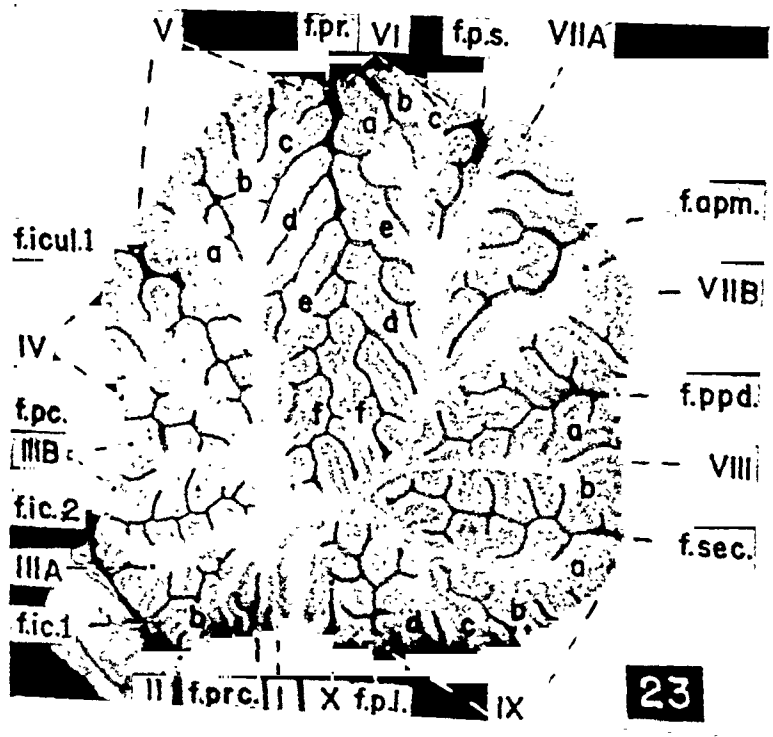
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which lived until near term, after having been subjected to castration prior to the appearance of prostatic buds and coagulating glands. A preliminary report of the observations was presented at the Providence meeting of the American Association of Anatomists (Wells, Cavanaugh and Maxwell, '52).

MATERIALS AND METHODS

In rats of the Sprague-Dawley strain, 113 pregnancies were timed by witnessing the mating. Forty-five yielded a closely graded series of control fetuses. Sixty-eight were used in the experiments on castration; a number of them also served as sources of control fetuses which were taken at the time of the operation.

At operation a gravid female was anesthetized with ether, and the uterus exposed by opening the peritoneal cavity at the linea alba. Each fetus to be castrated was brought into view by incising the uterus and fetal membranes, and was then delivered into the peritoneal cavity of the mother in such manner that the placental circulation remained intact ("extrauterinized fetus"). On each side of the fetal body an oblique inguinal incision was made, the testis removed and the wound closed by means of interrupted sutures of silk; for additional details of the method, two earlier papers may be examined (Wells, '50a; Wells and Fralick, '51, Method 2). In each of 4 cases a pellet of testosterone propionate (Perandren), weighing about 1.0 mg, was implanted under the fetal skin. In all cases the treated fetus, still attached to the uterus by the placenta and umbilical cord, was allowed to remain in the maternal peritoneal cavity. In each of three cases the fetus was covered, however, by means of a tent created by stitching a thin sheet of cellulose acetate to the uterine wall (artificial uterus). Similarly, in each of 4 cases, the fetus was covered by a tent which was fashioned out of a thin sheet of rubber and stitched to the uterine wall. When all steps of the fetal surgery were completed, the maternal wound was closed by suturing and the etherization stopped.

The experiments were terminated by killing the mothers. This was done near term and usually when the fetuses had attained the age of 21 days and 15 hours. It was found that 14 of the castrated fetuses were alive and quite active and that the "artificial uteri" had not increased the percentage of survival. The survival was as follows. Of two fetuses castrated at the age of about 19½ days, both were alive (littermates). Of 61 castrated at ages of 18 d + 2 h up to 18 d + 16 h, 12 were living. Of 15 castrated at 17 d + 21 h to 18 d + 1 h, none was alive.

In conducting the autopsy of an individual litter, a living castrated fetus and an unoperated male were taken by severance of the umbilical cords. They were promptly weighed and then killed. The reproductive tracts were fixed *in situ* in Bouin's solution, and sectioned transversely in perfect series at 10 μ. The sections were mounted on microscope slides and stained with hematoxylin and eosin.

With the aid of microscopes, data were obtained from the stained sections. The length of the Müllerian ducts and/or prostatic utricle was determined by simple calculation (number of transverse sections × thickness of section), on the assumption that they were straight vertical structures, which, of course, they were not; nevertheless the data would seem to have comparative value. Such data were not obtained in the case of the fetuses of group C (table 2) since the reproductive tracts had been sectioned in the frontal, rather than the transverse, plane. In 20 fetuses the counting of the prostatic buds was facilitated by making transparent models of the prostatic urethra and outgrowths. The models were prepared by projecting the sections with an Edinger projector, by stippling the desired outlines on thin sheets of cellulose acetate with inks of various colors and by stacking these sheets. The models were abnormally short in vertical dimension because the acetate sheets were thin and because suitable spacers of cardboard were deliberately omitted. The models were, however, accurate in two dimensions and in the number of prostatic buds shown.

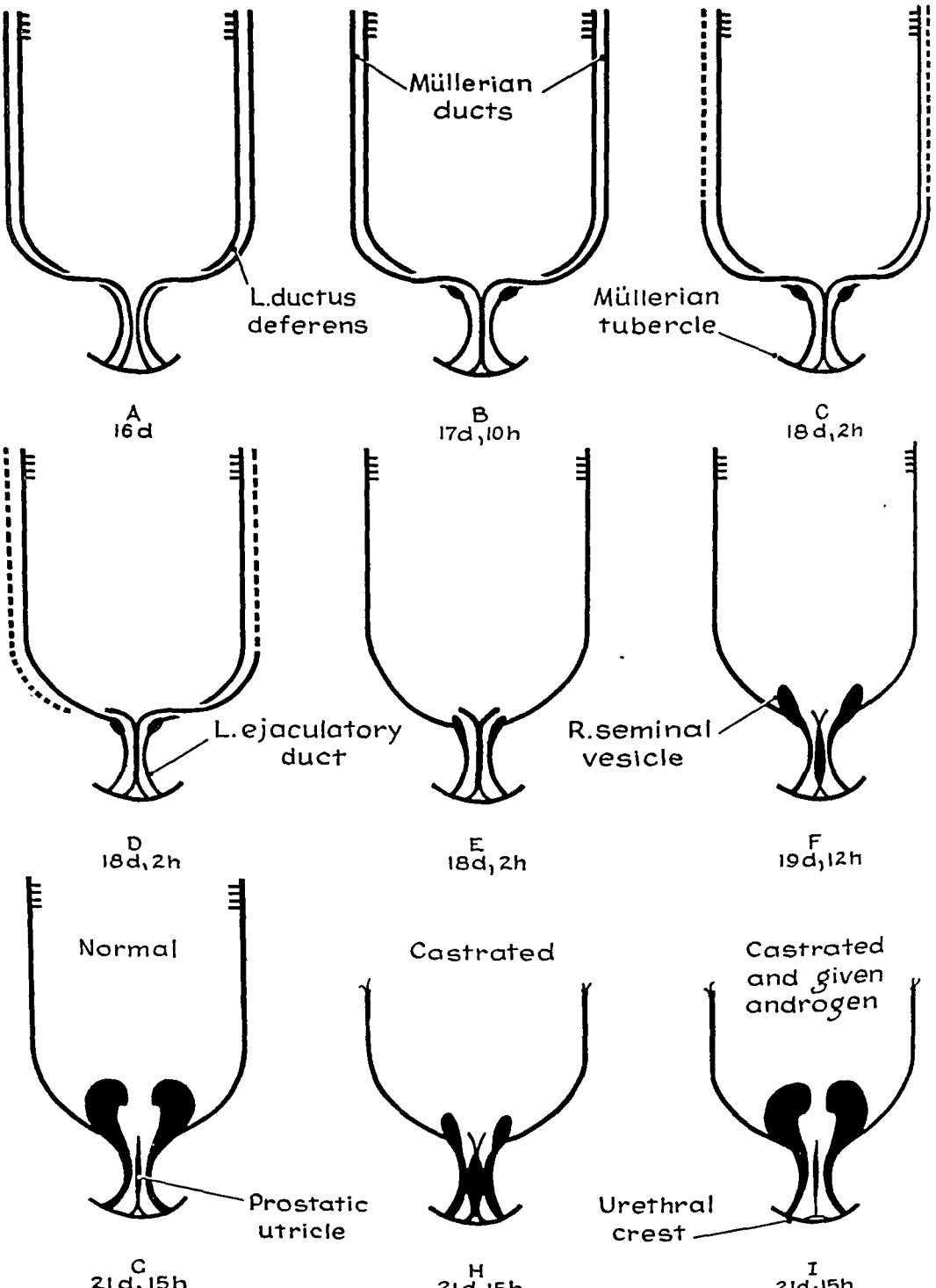


Fig. 1 Conventionalized diagrams of the genital ducts and derivatives in control fetuses (A to G) and experimental fetuses (H and I). At the age of 18 d + 2 h, the prevailing pattern was like that illustrated in drawing C (12 of the 14 fetuses). Only one of the castrated fetuses showed a "V" at the upper end of the prostatic utricle (drawing H).

TERMINOLOGY

The *coagulating glands,* most easily defined by reference to the adult rat, are those paired parts of the prostatic complex which are topographically associated with the seminal vesicles and which release into the urethra a substance that coagulates ejaculated semen to produce the "vaginal plug" Moore, '39, fig. 26). The *prostatic utricle* (uterus masculinus), homologue of the vagina in the female and derived from the Müllerian ducts, is present in the newborn male. The *Müllerian tubercle* in a fetus is that hillock in the urogenital sinus which receives the Müllerian ducts and Wolffian ducts (fig. 1 A) and which persists after birth as a mound (colliculus seminalis) on the urethral crest. The *balano-preputial lamella* is a solid plate of epithelium from which the epithelium of the glans penis and that of the inner surface of the prepuce develop (insert in fig. 17).

OBSERVATIONS

In preview it should be noted that in 6 of the 14 castrated fetuses to be considered the operation was performed at developmental stages younger than 18 d + 11 h (table 2). In almost all of the control males under 18 d + 11 h, the epithelial buds of the prostate and the epithelium of the coagulating glands had not yet appeared (table 1); the oblique and horizontal portions of the Müllerian ducts were still present (fig. 1 C, see text).

Control males

Prostate. In controls younger than 18 d + 11 h, there was only one instance in which a prostatic bud was found. This structure, noted in a fetus of 18 d + 4 h, appeared in only three of the 10 μ sections. It was situated in a region comparable to the one indicated by the arrow in figure 13. Up to and including the stage of 18 d + 14 h, the largest number of buds in an individual male was 6. Such buds were smaller and shorter than those illustrated in figure 16.

TABLE 1

Observations in 83 control fetuses [1]

FETUSES	AGE, DAYS + HOURS	BODY WT.	PREPUTIAL FRENULUM, WIDTH	MÜLL. DUCTS + PROST. UTRICLE, LENGTH	PROSTATIC UTRICLE, RL × DV [2]	SEMINAL VESICLES, EPITHELIUM [3]	COAGULATING GLANDS, EPITHELIUM	PROSTATIC BUDS, EPITHELIUM
		gm	*μ*	*μ*	*μ*			
2	16					A	0	0
2	16 + 12					D	0	0
2	16 + 16					D	0	0
3	17 + 10	.700				V (2)	0	0
1	17 + 12	.880				D	0	0
1	17 + 14	.716				V	0	0
1	17 + 18	1.035				V	0	0
1	17 + 20	1.087				V	0	0
1	17 + 21	.985				V	0	0
1	17 + 23					V	0	0
3	18					V (2)	0	0
1	18 + 1			980		V	0	0
14	18 + 2			1050	102 × 82	V (13)	0	0
13	18 + 3			940	107 × 89	V	0	0
7	18 + 4			880	102 × 84	V	0	1 [4]
5	18 + 5	1.257 (1)		890	111 × 82	V	0	0
3	18 + 7	1.274 (2)		720	127 × 73	V	0	0
1	18 + 11	1.650	458	860	116 × 78	V	0	4
2	18 + 12	1.434	429 (1)	1040	104 × 81	V	0	3 [4]
2	18 + 13	1.590		660	126 × 70	V	0	4 [4]
2	18 + 14	1.631	458 (1)	930	112 × 77	V	0	6
1	19 + 12	2.453		420	139 × 109	V	+	⩽ 20
1	19 + 14	2.487	415	480	153 × 95	V	++	⩽ 20
13	21 + 15	5.003	40 (12)	258 (12)	83 × 77	Vef	+	61 (6)

[1] Numbers in parentheses indicate the number of cases.
[2] RL, right-left diameter; DV, dorsal-ventral diameter.
[3] A, absent (Wolffian ducts not dilated at sites of future vesicles); D, dilatation of Wolffian ducts at sites of future vesicles; V, vesicular outgrowths; Vef, vesicles each with a "cranial flexure" (flexure illustrated in fig. 1).
[4] Actual number in one case (not an average number); no buds in the other cases.

In fetuses so young that the prostatic buds had not yet appeared, it was not possible to identify any well defined boundaries of the future prostate. Controls of 18 d + 2 h and older showed, however, in the region surrounding the epithelium of the future prostatic urethra, a relatively thick area of densely packed cells which must have originated from mesenchyme (fig. 13). That most of these cells are eventually used in forming the non-parenchymal component of the prostate (stroma, etc.), was suggested by the minute anatomy of the developing prostate in older fetuses (figs. 16 and 14).

The prostatic buds in fetuses of 21 d + 15 h were numerous. Distally, some of them had distinct branches (fig. 2). The longest buds were those of the ventral series.

Coagulating glands. The coagulating glands were observed in 15 of the controls (19 d + 12 h to 21 d + 15 h). They consisted of two components, inner and outer. The inner component, a solid core of epithelium, had originated from the dorsal aspect of the embryonic prostatic urethra (figs. 6 and 2). The outer component was a thick investment of densely packed cells of mesenchymal origin.

The question arose as to whether the primordia of the coagulating glands might exist, in advance of epithelial outgrowths, in the form of clusters of densely packed cells (e.g., primordia of the outer component of the glands). A positive answer was suggested by the fact that two cellular condensations were observed in each of the two fetuses of 18 d + 14 h and that they were situated in the region of the future coagulating glands, or near it. Also, they had undoubtedly originated from mesenchyme. Incidentally, they were smaller and less distinct than those marked by the arrows in figure 7.

In fetuses younger than 18 d + 11 h, there did not seem to be any paired cellular condensations in what was regarded as the region of the future coagulating glands (fig. 5).

Seminal vesicles. Vesicular outgrowths from the Wolffian ducts were regularly present in fetuses killed at an age of 18 d + 2 h, hence the developing vesicles need only be given brief consideration in the present paper. At 21 d + 15 h, the

original cranial end of each vesicle had a flexure which passed medially and inferiorly ("cranial flexure," fig. 1 G).

Bulbourethral glands. The primordia of the bulbourethral glands were not yet present in controls of 16 d to 16 d + 16 h. Either the primordia or the developing glands themselves were observed in all males of 17 d + 10 h to 21 d + 15 h.

Müllerian ducts and prostatic utricle. The normal retrogression of the Müllerian ducts in the male was already in progress in controls of 18 d + 2 h to 18 d + 14 h. The pattern usually observed in such fetuses is illustrated in figure 1 C. The stages shown in figures 1 D and 1 E were rarely seen. These observations suggested that in the castrated fetuses to be considered all, or almost all, of the vertical portions of the Müllerian ducts had already dropped out before the time of the operation.

In the prevailing pattern depicted in figure 1 C, the prostatic utricle had a lumen. At the Müllerian tubercle, the utricle ended by opening bilaterally into the urogenital sinus (embryonic prostatic urethra, fig. 13).

The prostatic utricle was present at 21 d + 15 h (figs. 1 G, 1 C and 14). Inferiorly, below the level of section shown in figure 10, the utricle usually had a small lumen which forked and then opened bilaterally into the urethra.

Penile urethra and preputial frenulum. In the fetuses of 18 d + 2 h to 19 d + 14 h the distal end of the urethra was a solid plate of epithelium situated in the developing glans penis (urethral plate, see insert in fig. 17). Just proximal to the urethral plate, the floor (ventral wall) of the urethra was still missing (open urethral groove, not illustrated in the photographs). The developing preputial frenulum was a ventral structure in the region where the right and left portions of the balano-preputial lamella were separated from each other by developing connective tissue. This frenulum was broadest proximally, at the site of the open urethral groove, and it was here that the data on the width of the frenulum were obtained.

TABLE 2

Observations in 14 castrated fetuses

GROUPS	FETUSES [1]	AGE AT OPERATION, DAYS + HOURS	EXPERIMENTAL PERIOD	BODY WEIGHT AT AUTOPSY	PREPUTIAL FRENULUM, WIDTH	MÜLL. DUCTS + PROST. UTRICLE, LENGTH	PROSTATIC *UTRICLE*, RL × DV [2]	COAGULATING GLANDS, EPITHELIUM		PROSTATIC BUDS, EPITHELIUM
								Absent	Present	
			hours	*gm*	*μ*	*μ*	*μ*			
Castrated fetuses not given androgen										
A	5E	18 + 6 [3–9]	82 [79–84]	4.408	517 (4)	596	225 × 94	(4)	(1)	10 [5–16]
	5C			5.442	37	253	81 × 81		(5)	65 (3)
B	5E	18 + 13 [11–15]	75 [74–77]	4.509	406	340	135 × 79		(5)	15 [9–20]
	5C			5.010	41	232	82 × 78		(5)	48 (1)
C	2E	19 + 8	61	3.178					(2)	26 [25–27]
	1C			3.211					(1)	55
Castrated fetuses given androgen										
D	2E	18 + 7 [2–13]	78 [74–85]	4.114	41	500	113 × 102		(2)	60 [59–62]
	2C			5.289	46	340	80 × 66		(2)	66 (1)

[1] Age of the fetuses at autopsy was about 21 d + 15 h. Numbers in parentheses indicate the number of cases. Numbers in brackets indicate the range. E, experimental; C, control (litter-mate).

[2] RL, right-left diameter; DV, dorsal-ventral diameter.

At 21 d + 15 h the urethra was a continuous tube. The urethral plate had acquired a lumen, and the earlier urethral groove had been closed by the union of the right and left urethral folds.

Castrated fetuses not given androgen (groups A to C, table 2)

Prostatic buds. Epithelial buds of the prostate were observed in each of the 12 fetuses of groups A to C. All buds except one to 4 of the ventral series were relatively short (fig. 3). The buds were usually unbranched. In each of three exceptional fetuses, one of the ventral buds was beginning to branch (see fig. 15).

Despite the fact that in a normal fetus taken from a non-experimental litter at 18 d + 4 h a single bud was already present (table 1), the data seemed to indicate that in each of the castrated fetuses of group A all of the prostatic buds had appeared subsequent to the orchiectomy. To illustrate what was found in individual litters, three fetuses of litter 941 may be used. A normal control, killed and preserved at the beginning of the experiment, 18 d + 7 h, had no buds. A second fetus, castrated at 18 d + 7 h, and killed at 21 d + 15 h, had 7 buds. The third, an unoperated control killed at 21 d + 15 h, had many buds (the buds could scarcely be counted accurately since a model was not made).

Similarly, the data indicated that in each of the castrated fetuses of group B most of the buds had appeared after the time of the operation. The findings in one litter, no. 932, may be presented. A normal control, killed at the beginning of the experiment, 18 d + 12 h, showed three buds. A second fetus, castrated at 18 d + 12 h and killed at 21 d + 15 h, showed 16 buds. The third, an unoperated control killed at 21 d + 15 h, showed 48 buds.

The buds in the castrated fetuses of group C were longer and more numerous than those of a normal fetus from which figure 16 was obtained. This fact together with the tabulated data seemed to show that any buds existing at the time of

the operation had continued to grow during the experimental period and also that some new buds had originated during this period.

Coagulating glands. In regions comparable to those where the coagulating glands of controls were observed, only paired clusters of cells of mesenchymal origin were found in 4 of the 5 castrated fetuses of group A (fig. 7). It was problematical whether these clusters constituted primordia of the outer, non-epithelial component of the glands.

The coagulating glands were present in 6 of the castrated fetuses of groups A and B. The tabulated data were regarded as ample evidence that the epithelial component of the glands had developed after the orchiectomy.

The glands were likewise present in the two castrated fetuses of group C. It was likely that these glands had already appeared at the time the operation was performed.

Müllerian ducts and prostatic utricle. The Müllerian ducts, as such, had largely disappeared, and were mainly represented by a large derivative, the prostatic utricle (fig. 1 H). That retrogression of the Müllerian ducts which had occurred during the experimental period was approximately the same in each castrated fetus of groups A and B; it usually proceeded from a stage shown in figure 1 C up to that shown in figure 1 H. In the castrated fetuses of group C such retrogression had already occurred, however, at the time of the operation, or shortly thereafter (fig. 1 F).

The prostatic utricles of the castrated fetuses were larger than those of the litter-mate controls of the same age (fig. 1 H, fig. 1 G and table 2). Utricles of the castrated fetuses of group A were the largest of all (fig. 7), even larger than those of two normal males which were killed at 19 d + 12 h and 19 d + 14 h, respectively. The utricles of the castrated fetuses of groups B and C were only somewhat larger than those of the litter-mate controls obtained at 21 d + 15 h.

The distal end of the utricle had the shape of an inverted Y in each castrated fetus, and, in this respect, was normal.

It was abnormal in each of the castrated fetuses of groups A and B in that the lumen was missing.

Müllerian tubercle and ejaculatory ducts. The Müllerian tubercle was usually abnormal in shape, being so flat that in section it scarcely resembled a hillock. The ejaculatory ducts also were usually abnormal. In each of the castrated fetuses of groups A and B, the distal end of each duct lacked a lumen. In 7 of these 10 fetuses the ducts were dilated, either bilaterally (5 fetuses) or unilaterally (2 fetuses); sometimes the corresponding seminal vesicles were also dilated. In each of two fetuses of group A, though, at a region well above the Müllerian tubercle, the ejaculatory ducts and the seminal vesicles were more or less solid structures (lumina missing or almost missing, figs. 11 and 15).

Penile urethra and preputial frenulum. The penile urethra was normal in one respect but abnormal in a second. It was normal in that the urethral plate had undergone cavitation to produce the fossa navicularis. It was abnormal in that the urethral groove was still open (hypospadias). The existence of a condition of hypospadias was actually observed in only 8 of the 12 castrated fetuses (three in group A, three in B and two in C), and was, for one reason or another, doubtful in the other 4. This anomaly was regarded as an abnormal persistence of an earlier normal embryonic condition. The proximal part of the developing preputial frenulum was usually very wide, thus reflecting the fact that the floor of the urethra was missing in this region.

Castrated fetuses given androgen (group D, table 2)

In each of the two experimental fetuses of group D, a pellet of testosterone propionate was implanted under the skin immediately after the testes had been removed. When the stained sections were studied microscopically, it was found that in each case all those effects of castration noted in the experimental fetuses of groups A to C had been prevented,

or largely prevented. Thus, the prostatic buds were numerous (fig. 4 and table 2). The coagulating glands were present (figs. 4 and 8). The ejaculatory ducts had continuous lumens, and were not abnormally dilated (fig. 12). The floor of the penile urethra was present (absence of hypospadias). The primitive preputial frenulum was narrow (fig. 20).

The effects of castration upon the prostatic utricle were largely prevented. The utricles of the experimental fetuses in group D were smaller than those of fetuses which had been castrated for a similar period of time and which had not been given androgen (figs. 11 and 12). The utricles were, however, somewhat larger than those of the unoperated littermates of the same age (table 2). At the Müllerian tubercle there was a lumen in the utricle of each fetus in group D; the tubercle and associated ducts were essentially like those illustrated in figure 14.

DISCUSSION

The genital abnormalities in the castrated fetuses included a reduction of the number of prostatic buds, absence of the coagulating glands, incomplete formation of the floor of the penile urethra (hypospadias) and enlargement of the prostatic utricle. The first three of these abnormalities could be prevented by means of testosterone propionate, and the 4th largely prevented.

Should these observations be regarded as evidence that in normal development the testicular androgen is a causative factor in the formation of the primordia of the male accessory reproductive organs? We would answer in the negative despite the absence of the coagulating glands in 4 of the castrated fetuses and despite the prevention of this anomaly by implanted testosterone propionate; such observations on the coagulating glands, though intrinsically sound, must be balanced against the current lack of demonstration that the fetal testis produces testosterone propionate. Also, a negative answer is suggested by the existence of prostatic buds

in castrated fetuses in which the operation had been performed prior to the time of expectation of buds.

A second question is whether the observations should be regarded as evidence that in normal development the testicular androgen is an important factor in the retrogression of the Müllerian ducts in the male. We have arrived at a negative answer. All castrated fetuses of group A showed a lack of persistence of those oblique and horizontal segments of the right and left ducts which must have existed at the time these fetuses were subjected to castration (fig. 1 C). Also, the enlargement of the prostatic utricle and the prevention of this effect by testosterone propionate must be considered in the light of what was found in the case of the ejaculatory ducts. In most of the experimental fetuses of groups A and B, the ejaculatory ducts were dilated. Such dilatation could be prevented by testosterone propionate.

Although a satisfactory explanation of the enlargement of the prostatic utricle and of the simultaneous dilatation of the ejaculatory ducts is not apparent, clues are afforded in the fact that distally these structures lacked lumens and that in two instances the seminal vesicles were somewhat dilated. It occurs to us that the utricles and ejaculatory ducts, as a consequence of lack of patency distally, might have been expanded at some time during the experimental period by an abnormal accumulation of fluid in their cavities. If so, it is reasonable to suppose that the testosterone propionate prevented such expansion by keeping open, in some indirect manner, the orifices of the utricles and ejaculatory ducts.

The observed hypospadias, explained embryologically as an abnormal persistence of the embryonic urethral groove, is undoubtedly a consequence of the orchiectomy. On the other hand, it may not be due solely to the experimental deprivation of testicular androgen. Its prevention by testosterone propionate does not entirely rule out the possibility that some non-specific "physiological insult" during the experimental surgery might have acted as a contributing factor. Other workers have found that hypospadias and other genital anoma-

lies may be produced experimentally in the male offspring of rats by feeding the gravid female a diet deficient in vitamin A (Wilson and Warkany, '48).

Our data support the view that in rats testicular androgen is produced before birth and that it stimulates the prenatal growth of the male accessory reproductive organs (Wells, '50b; Wells and Fralick, '51; see Maxwell and Wells, '51). This view, incidentally, is also supported by the fact that when the fetal testis is grafted into the seminal vesicle of an adult castrated host it is capable of producing androgen at once (Jost, '48; Moore, '53).

The reduced number of prostatic buds in our series of castrated fetal rats is at variance with certain observations in young opossums (Moore, '50a, '50b). After the removal of the testes of an opossum on the 20th day of life the existing prostatic buds (about a half-dozen) continue to grow, new buds are rapidly added and the buds undergo extensive branching; up to the 100th day the prostate of a castrated opossum is similar to that of a normal control. The present authors do not know why there should have been such a discrepancy in the results obtained in these two species of mammals, rat and opossum (placental mammal vs. marsupial). On the other hand, the lack of persistence of the Müllerian ducts in our castrated fetuses and also the actual formation of prostatic buds during the experimental period are in keeping with similar observations in the castrated opossums (Moore, '50b).

Absence of the coagulating glands and the presence of hypospadias in our rats are anomalies like those found in fetal mice in which the testes were destroyed by irradiation (Raynaud and Frilley, '48; Raynaud, '50). This castration by irradiation was performed on the 13th day after conception, and the fetal mice were killed on the 18th day. The abnormalities, more extensive than those in our specimens, included absence of the seminal vesicles and prostate and, incidentally, the presence of an epithelial cord derived from the Müllerian ducts ("cordon vaginal"). Raynaud ('50) states that the

genital tubercle of a castrated mouse was structurally similar to that of a normal female control. The authors did not present a record of having prevented these effects by means of introduced androgen.

The complex of abnormalities noted in our material is less extensive than that found in fetal rabbits (Jost, '50). In rabbit fetuses castrated on the 19th to 21st day of life and killed on the 28th day, Jost reported absence of the prostate and the differentiation of the Müllerian ducts into a vagina (prostatic utricle ?) and into certain segments of uterine horns and tubes. Such effects in rabbit fetuses were prevented by implanted testosterone propionate.

In our work, the formation of prostatic buds in the absence of testes is in harmony with the existence of "female prostates" in adult female rats. Prostates occur spontaneously in a high percentage of the females in certain selected strains of inbred rats (Price, '44).

It is clear that the enlargement of the prostatic utricle in the castrated fetal rat is attributable to the castration. It is problematical, however, whether this change is due to the experimental deprivation of testicular androgen, as was implied earlier in the discussion (paragraphs 3 and 4). This over-all interpretation is supported by the fact that in the rhesus monkey, for example, the introduction of androgen into the circulation of a gravid female fails to cause in a female fetus any retrogression of the Müllerian ducts and derivatives but does produce a high degree of intersexuality (Wells and van Wagenen, in press). Our over-all interpretation was formulated in spite of certain observations in chick embryos and in a single rabbit fetus. In a female chick embryo in which a developing testicle has been grafted into the peritoneal cavity, the graft may bring about a complete retrogression of the Müllerian ducts of the host (Wolff, '50). Similarly, when an embryonic testis is grafted onto the somatopleure (a membrane which later participates in the formation of the chorioallantoic membrane) of a female chick embryo, the graft may cause a complete disappearance of the Mül-

lerian ducts (Huijbers, '51). Also, in a single case of a female rabbit fetus in which a testicle had been grafted onto the developing mesosalpinx, it was found that a segment of the Müllerian duct was missing on that side of the body (Jost, '50).

In closing the discussion, our data bring into sharp focus the question of whether in normal male fetuses the testicular androgen is produced early enough to, and at such early stages in sufficient quantity to, induce the formation of the primordia of "male organs" and to cause the normal retrogression of the Müllerian ducts. We think this is an open question.[3]

SUMMARY AND CONCLUSIONS

Fetal rats so young that they still lacked prostatic buds and coagulating glands and so young that they still had long segments of the Müllerian ducts, were subjected to castration and then permitted to live until near term.

It was found that prostatic buds had appeared during the experimental period but that the number of buds was significantly smaller than the number in controls of the same age and litter. The coagulating glands were sometimes absent. The floor of the penile urethra was usually missing (hypospadias). All portions of the Müllerian ducts except the prostatic utricle had disappeared. The utricle was relatively large.

These effects of castration could be prevented, or largely prevented, by means of implanted testosterone propionate.

Our observations support the view that in rats testicular androgen is produced before birth and that it stimulates the prenatal growth of the male accessory reproductive organs. These observations are inadequate to determine, however,

[3] It would be beyond the scope of the present paper to undertake a review of the vast literature on the role of hormones in sex differentiation in mammals. The whole subject was considered at a recent Colloquium; the Proceedings of this symposium appeared serially in 1950 (Arch. d'Anat. micr. Morph. exp., 39), and the papers were assembled under one cover in 1951 (La Différenciation Sexuelle chez les Vértébres, Centre National de la Recherche Scientifique, Paris 7).

whether androgen from the fetal testis acts as a causative factor in the formation of the primordia of these organs and in the normal retrogression of the Müllerian ducts.

LITERATURE CITED

HUIJBERS, M. M. 1951 De invloed van de gonade op de ontwikkeling van het geslachtsapparaat bij het kippenembryo. Thesis, Amsterdam. Abstracted in English in 1952: Excerpta Medica, 6 (sect. 1): 34–35.

JOST, A. 1948 Activité androgène du testicule foetal de rat greffé sur l'adulte castré. Compt. rend. Soc. de Biol., *142:* 196–198.

———— 1950 Sur le contrôle hormonal de la différenciation sexuelle du lapin. Arch. d'Anat. micr. et de Morph. exp., *39:* 577–607.

MAXWELL, E. L., AND L. J. WELLS 1951 Effects of grafted testes upon the reproductive organs of castrated fetal rats. Anat. Rec., *109:* 378 (abstract).

PRICE, DOROTHY 1944 The reactivity of the prostate gland of the female rat in relation to age. Am. J. Anat., *75:* 207–231.

RAYNAUD, A. 1950 Recherches expérimentales sur le développement de l'appareil génital et le fonctionnement des glandes endocrines des foetus de souris et de mulot. Arch. d'Anat. micr. et de Morph. exp., *39:* 518–576.

RAYNAUD, A., AND M. FRILLEY 1948 Effet, sur le développement des diverses glandes annexes du tractus génital des embryons de Souris, de la destruction, au moyen des rayons X, des glandes génitales des embryons. Compt. rend. Soc. de Biol., *142:* 422–426.

MOORE, C. R. 1939 Biology of the Testes. Sex and Internal Secretions. Ed. by E. Allen, C. H. Danforth and E. A. Doisy. Williams and Wilkins Co., Baltimore. Chap. VII, 353–451.

———— 1950a Studies on sex hormones and sexual differentiation in mammals. Arch. d'Anat. micr. Morph. exp., *39:* 484–498.

———— 1950b The role of the fetal indocrine glands in development. J. Clin. Endocrinol., *10:* 942–985.

———— 1953 Adrenal cortical secretions in relation to the reproductive system of rats. J. Clin. Endocrinol. and Metabol., *13:* 330–368.

WELLS, L. J. 1950a Subjection of fetal rats to surgery and repeated subcutaneous injections: method and survival. Anat. Rec., *108:* 309–331.

———— 1950b Hormones and sexual development in placental mammals. Arch. d'Anat. d, micr. Morph. exp., *39:* 499–517.

WELLS, L. J., M. CAVANAUGH AND E. L. MAXWELL 1952 Development of the reproductive organs in castrated fetal rats: prostate, coagulating glands, genital papilla and prostatic utricle. Anat. Rec., *112:* 467 (abstract).

WELLS, L. J., AND R. L. FRALICK 1951 Production of androgen by the testes of fetal rats. Am. J. Anat., *89:* 63–107.

WELLS, L. J., AND G. VAN WAGENEN Androgen-induced female pseudohermaphroditism in the monkey *(Macaca mulatta):* anatomy of the reproductive organs. Contrib. to Embryol., Carnegie Inst., Wash., *35:* (in press).

WILSON, J. G., AND J. WARKANY 1949 Malformations in the genito-urinary tract induced by maternal vitamin A deficiency in the rat. Am. J. Anat., *83:* 357–395.

WOLFF, E. 1950 Le rôle des hormones embryonaires dans la différenciation sexuelle des oiseaux. Arch. d'Anat. micr. Morph. exp., *39:* 427–450.

PLATE 1

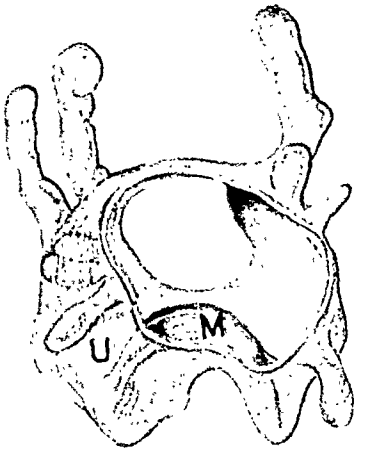
EXPLANATION OF FIGURES

The drawings depict the urethra (U) and prostatic buds as they appeared, when viewed from above, in transparent models which were prepared by stippling onto sheets of cellulose acetate the configuration of the epithelium (see text). The orientation is the same as that shown in figure 14, i.e., dorsal aspect down and right aspect on the observer's right. At the Müllerian tubercle (M), the prostatic utricle and the ejaculatory ducts were not modeled. C, coagulating gland.

2 Unoperated control of 21 d + 15 h. Litter 966. There were 61 prostatic buds, some of which are hidden in the drawing. ×75.

3 Fetus castrated at the age of 18 d + 3 h and killed at the age of 21 d + 15 h. Litter 966. There were 9 prostatic buds, all shown in the drawing. The coagulating glands were absent, as illustrated. × 75.

4 Fetus castrated at 18 d + 2 h, given a pellet of androgen at 18 d + 2 h and killed at 21 d + 15 h. Litter 967. There were 62 buds (66 buds in an unoperated litter-mate of the same age). × 75.



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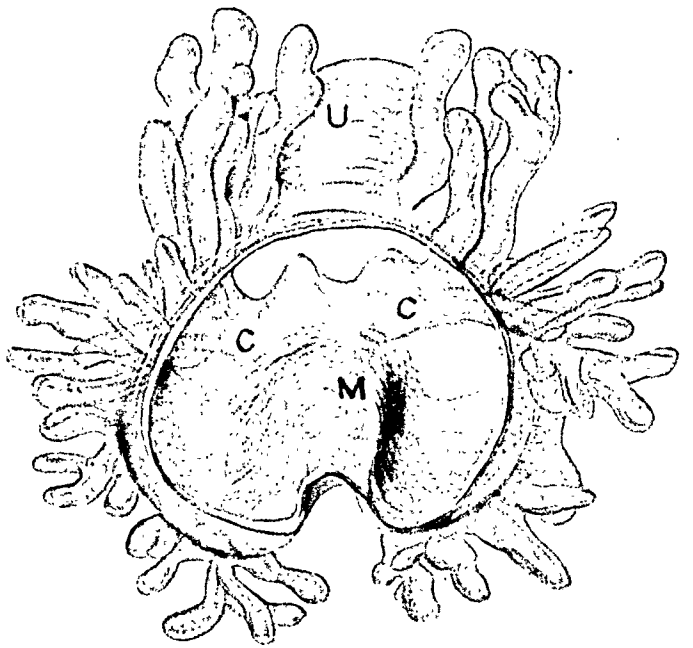

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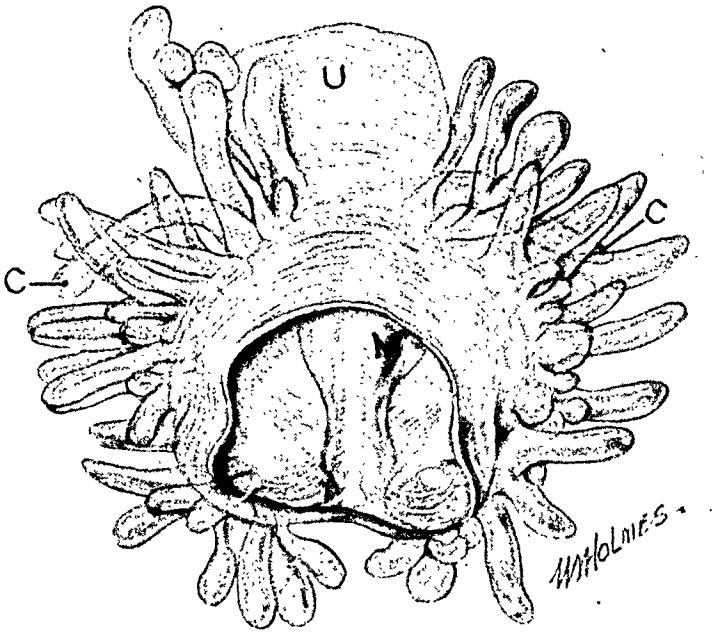

PLATE 2

EXPLANATION OF FIGURES

Eight transverse sections of the pelvic region of several fetuses presented for the purpose of illustrating the prostatic utricle, ejaculatory ducts, coagulating glands and prostatic buds.

Each section is oriented in such manner that the dorsal aspect is down (note rectum) and the right aspect on the observer's right. Ureters and wall of bladder in figures 5 and 9. Urethra in 6 of the illustrations (up — figs. 6–8 and 10–12).

The prostatic utricle appears in each illustration except figure 6 (near center of photo). Right and left ejaculatory ducts in 5 of the photos (figs. 7 and 9–12); right duct in three (figs. 5, 6 and 8). Left ductus deferens and the duct of the left seminal vesicle in figures 6 and 8.

5 Normal male fetus of 18 d + 2 h. Litter 1346. × 60.

6 Unoperated control of 21 d + 15 h. Litter 966 (cf. fig. 2). Prostatic utricle not shown since it was below the level of the transverse section presented (see figs. 10 and 1 G). Coagulating glands present (black objects in top third of photo). × 60.

7 Fetus castrated at 18 d + 3 h and killed at 21 d + 15 h. Litter 966. Prostatic utricle large. Ejaculatory ducts dilated. Coagulating glands missing (epithelium missing). Arrows indicate two masses of cells in a region where the coagulating glands might be expected (see text). × 60.

8 Fetus castrated at 18 d + 2 h, given androgen at 18 d + 2 h and killed at 21 d + 15 h. Litter 967. Coagulating glands present. × 60.

9 Normal male of 18 d + 2 h. Litter 1335. × 60.

10 Unoperated control of 21 d + 15 h. Litter 953. White arrow, epithelium of the prostatic utricle. Black arrow, outer margin of utricle. Cavity of utricle not shown in the section presented. × 60.

11 Fetus castrated at 18 d + 4 h and killed at 21 d + 15 h. Litter 953. Left ejaculatory duct solid, right duct almost solid. × 60.

12 Fetus castrated at 18 d + 2 h, given androgen at 18 d × 2 h and killed at 21 d + 15 h. Litter 967. Photo supplements figure 8 by showing additional prostatic buds (see fig. 4). × 60.

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Four transverse sections of the pelvic region. Orientation: dorsal aspect down (note rectum) and right aspect on observer's right.

13 Normal fetus of 18 d + 2 h, obtained at beginning of experiment. Litter 941. Urethra in oblique section. Absence of prostatic buds. Arrow marks approximate site where a single bud was found in another fetus (normal fetus of 18 d + 4 h, table 1). White blister on the right aspect of the Müllerian tubercle is the prostatic utricle (cf. fig. 1, drawing C). × 50.

14 Unoperated control obtained at end of experiment, 21 d + 15 h. Litter 941. Prostatic urethra, numerous prostatic buds, Müllerian tubercle, bilateral termination of prostatic utricle (marked by two black ink dots) and termination of the ejaculatory ducts (sites about 6 mm below ink dots). × 50.

15 Fetus castrated at 18 d + 7 h and killed at 21 d + 15 h. Litter 941. Prostatic urethra, a small number of prostatic buds (total of 7 buds in model), prostatic utricle (marked by the two white ink dots) and ejaculatory ducts (absence of lumen in right duct). × 50.

16 Normal fetus of 19 d + 12 h. Prostatic urethra and buds (19 buds counted in the serial sections), and a portion of the Müllerian tubercle. × 60.

Five sections of the developing penis (photos obtained from transverse sections of 5 fetuses). Orientation: dorsum of penis on observer's left; left aspect down.

17 (Large photo.) Normal fetus of 18 d + 2 h. Litter 1355. Asterisk indicates left preputial gland. Photo also shows the cavernous bodies of the penis and the penile urethra (latter sectioned dorsal to the region of the urethral groove — groove not illustrated). × 30.

17 (Inserted small photo.) Normal fetus of 18 d + 2 h. Litter 1335. Glans penis and the prepuce are attached to each other by the balano-preputial lamella. Note urethral plate (median solid epithelium). × 20.

18 Unoperated control of 21 d + 15 h. Litter 966. Preputial glands (observer's left), glans penis and attached prepuce, penile urethra and a narrow preputial frenulum (latter on observer's right). × 30.

19 Fetus castrated at 18 d + 3 h and killed at 21 d + 15 h. Litter 966. Glans penis, prepuce and penile urethra (latter on observer's right). The section presented does not pass through the existing (abnormally persisting) urethral groove. × 30.

20 Fetus castrated at 18 d + 2 h, given androgen at 18 d + 2 h and killed at 21 d + 15 h. Litter 967. Narrow preputial frenulum (observer's right). × 30.


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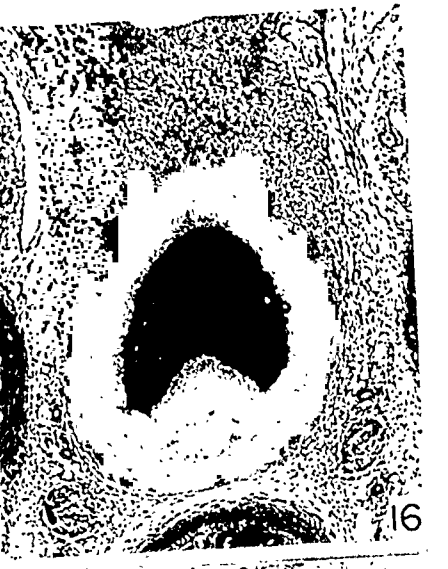
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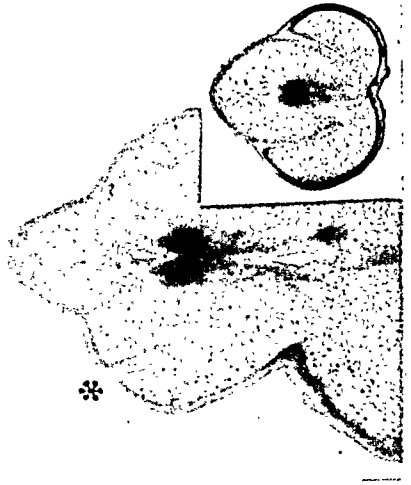
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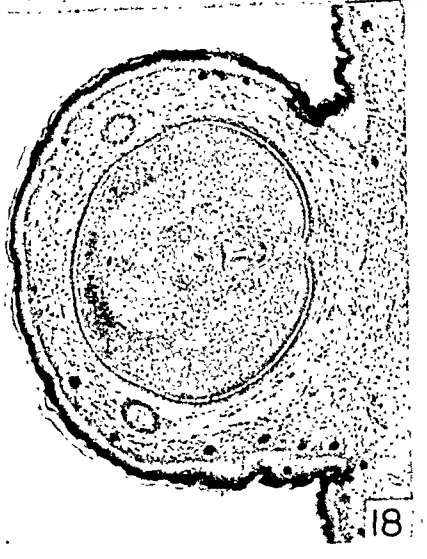
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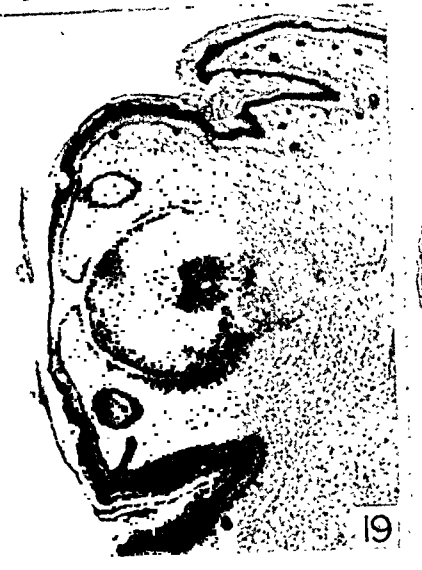
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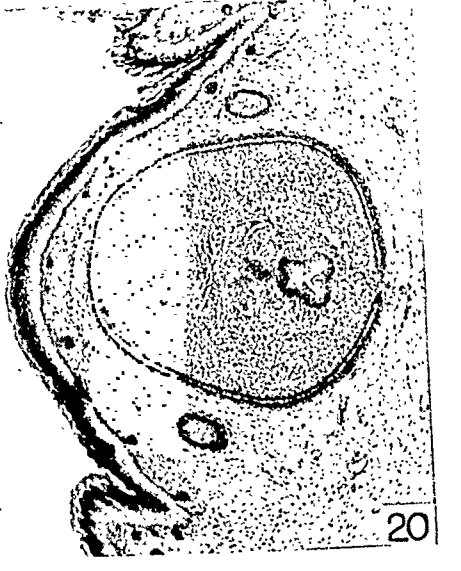
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THE IDENTIFICATION, ORIGIN, AND MIGRATION OF THE PRIMORDIAL GERM CELLS IN THE MOUSE EMBRYO

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SIX FIGURES

INTRODUCTION

The extra-gonadal origin of the primordial germ cells and the existence of the so-called "Keimbahn" or germ-tract is today accepted by most investigators. Germ cells have been identified and traced in numerous vertebrate embryos and their existence also demonstrated by experimental procedures, particularly in amphibian and bird embryos (see the reviews of Heys, '31; Everett, '45; Niewkoop, '49). But in mammalian embryos the existence of germ cells has been claimed by some and denied by others. This difference of opinion arises from a questioning of the validity of the criteria employed to distinguish germ cells from somatic cells. In mammalian embryos the germ cells are usually characterized as being larger than surrounding cells and as possessing a prominent nucleolus and more darkly staining nuclear and plasma membranes. On the basis of such morphological characteristics, the origin and migration of germ cells have been described in mammals (Vanneman, '17; Rubaschkin, '08, '09) and particularly in human embryos (Fuss, '12; Politzer, '33; Hamlett, '35; Witschi, '48). Other investigators, however, have found cells with these same characteristics so widely distributed throughout the tissues of the embryo that

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they have concluded either that primordial germ cells do not exist (Hargitt, '25) or that they cannot be identified by these criteria (Simkins, '23, '28). There is, therefore, a great need for a technique which will specifically stain the germ cells and permit them to be distinguished from all other cells.

We have found it possible to stain the primordial germ cells specifically with the Gomori histochemical technique for alkaline phosphomonoesterase activity. The origin of the germ cells in 8-day mouse embryos and their distribution in 9- and 12-day embryos will be described. From these observations it can be concluded that the primordial germ cells in the mouse embryo have an extra-gonadal origin and that they migrate along a germ-track to attain their final location within the germinal ridges.[2]

MATERIALS AND METHODS

This paper is based upon the study of Swiss albino mouse embryos ranging in age from 6 to 12 days *post coitum*. More than 50 embryos have been sectioned serially and studied. The methods used in the timing and collecting of embryos have been previously described (Chiquoine, in press). Throughout this paper, the ages of the embryos will be consistently stated in terms of days of copulation age.

Embryos were fixed in 80% alcohol, absolute acetone, or 5% acetic acid in absolute alcohol (Wolman and Behar, '52) at a temperature of —5° to —10°C. Acetone fixation was found to be slightly inferior to alcohol and to the acetic acid-alcohol mixture. Embryos of 6- to 10-day copulation age were fixed *in utero*, while the older embryos were dissected from the fetal membranes prior to fixation. All embryos were embedded in paraffin (Fisher, Tissuemat) and serially sectioned. The embryos were sectioned and tested for phos-

[2] Following the publication of a preliminary abstract of this study (Chiquoine, '53), Dr. J. S. Baxter informed me in a letter of his own observations on the staining of primordial germ cells in a 10-mm human embryo with the Gomori alkaline phosphatase technique. Doctor Baxter's observations were reported at the international Anatomical Congress at Oxford in 1950 but the abstracts of the papers presented at this meeting have not been published to date.

phatase activity on the same day in order to minimize any loss of enzyme activity from the spread sections.

The serial sections were run through the procedures for the demonstration of alkaline phosphatase activity according to the revised method of Gomori ('46). Sodium glycerophosphate (Kodak no. 644) was used as a substrate, buffered to pH 9.4. The serial sections were incubated at 37°C. in the buffered substrate for a period of one-half to one hour. Enzymatic activity was visualized as a black precipitate of cobalt sulphide. Control series were incubated in the same buffered mixture but lacking substrate.

OBSERVATIONS

The identification of the germ cells. Germ cells can easily be identified by virtue of their high alkaline phosphatase activity. Following the Gomori histochemical technique the cells are stained black by a precipitate of cobalt sulphide while the surrounding cells show only a minimal or negative reaction. The selective staining of the germ cells, however, is only possible when a short incubation period in the buffered substrate is employed. Longer incubation periods result in a positive reaction in almost all cells of the embryo and a corresponding decrease or complete lack of contrast between germ cells and somatic cells. The best contrast is obtained in the older embryos since the surrounding cells at these stages possess less enzymatic activity than the cells surrounding the germ cells in the younger embryos.

The staining of the germ cells can be attributed to enzymatic activity since the cells are unstained in the control series.

The enzymatic activity of the germ cells is observed in most cases to be localized in the cytoplasm, but in some cases both the cytoplasm and the nucleus appear to be stained black. The apparent staining of the nucleus can frequently be attributed to viewing the nucleus through an overlying, positively stained cytoplasm (fig. 5).

The selective staining of the germ cells permitted them to be counted, a procedure which is difficult and open to severe

criticism when such counts are made without the benefit of selective staining. An additional advantage of the series studied is the possibility of following those germ cells which stray from the principal migratory pathway or germ-track, no matter how far afield they may wander.

The origin of the germ cells. No primordial germ cells could be identified in 6- or 7-day embryos. This negative finding is based upon the careful searching of complete serial sections of more than 20 embryos.

The primordial germ cells are first recognized in 8-day embryos and are seen scattered among the cells of the caudal end of the primitive streak, the root of the allantoic mesoderm, and the underlying yolk sac splanchnopleure (figs. 1, 2, 3). The cells are confined to the mid-sagittal region of the embryo and do not extend laterally in the primitive streak or on to the surface of the yolk sac to any appreciable extent. Within the splanchnopleure of the yolk sac the germ cells typically occur as individual cells with entodermal or mesodermal cells separating adjacent germ cells. In the primitive streak and especially in the allantoic mesoderm the germ cells are more frequently observed in groups. The contrast between the germ cells and somatic cells is much better in the yolk sac than in the primitive streak or allantoic mesoderm because the somatic yolk sac cells exhibit less enzymatic activity than the somatic cells in the primitive streak and allantois.

Migratory pathway of the germ cells. The migratory pathway of the primordial germ cells in the mouse embryo is essentially similar to that described in the human embryo (Witschi, '48).

Subsequent to their origin in the caudal end of the embryo in early 8-day embryos, the germ cells migrate caudally and ventrally on to the yolk sac and caudally into the allantoic mesoderm. In 9-day embryos the cells have migrated from these extra-embryonic regions to the embryo proper. The cells are observed in the lateral and caudal face of the open mid gut, in the stalk of the allantois, and in the splanchno-

pleure of the hind gut. Within the hind gut the cells are located most commonly in the lateral and ventral portions of the gut wall. The quality of fixation resulting from the use of fixatives designed primarily to preserve enzymatic activity makes it difficult to determine in all instances whether a given germ cell is located within the entodermal epithelium or in the adjacent splanchnic mesoderm. However, many cases are clearly recognized of each position so that it is quite clear that the cells are not limited to either germ layer, but migrate freely within the splanchnopleure.

In 10-day embryos the germ cells are still found in the splanchnopleure of the gut, caudal to the open mid gut, but not extending as far caudally into the cloacal regions as they did in 9-day embryos. In a few series, two or three germ cells may still be observed in the open mid gut region but this is exceptional. In addition to their distribution in the splanchnopleure of the gut, the cells are also present in the dorsal mesentery, its root, and the adjacent germinal ridges (fig. 4). Within the germinal ridges the few germ cells present are located beneath the germinal epithelium, although in a few instances they form an integral part of this epithelium.

The distribution of germ cells in 11- and 12-day embryos is quite similar to the distribution in 10-day embryos, but the relative numbers of cells at the different loci have changed. Briefly summarized these changes are: an increasing number of germ cells at the end of the germ-track, i.e., within the germinal ridges, and a decreasing number of cells in the earlier portions of the germ-track, i.e., within the splanchnopleure of the gut and the dorsal mesentery. In late 12-day embryos the migration of the germ cells is practically completed. A few cells are still observed in the root of the dorsal mesentery but the vast majority of them are concentrated within the germinal ridges. Within the ridges the cells are located both in and beneath the germinal epithelium. The majority of the germ cells are situated beneath the epithelium for the number of germ cells within the epithelium is not more than 10% of the total number of germ cells in the ridges

(fig. 6). Thus in the late 12-day embryo the primordial germ cells have attained their definitive position. The later differentiation of the gonads and the relation of the germ cells described here to the functional gametes of the sexually mature mouse is the subject of further investigation.

During the course of their migration, only a few germ cells deviate to so great an extent from the described pathway that they can be considered as lost and perhaps incapable of ever reaching the germinal ridges. Such cells have been identified in numerous locations, viz., in the mesenchyme about the cloaca, within the mesonephric tubules, and in the developing wall of the dorsal aorta. It is of course possible that even these cells would eventually reach the germinal ridges. On the other hand, the cells are not observed to stray cephalad of the level of the germinal ridges nor into any entodermal or ectodermal organs such as neural tube and its derivatives, pancreas, or liver.

The number of germ cells increases quite rapidly with development, but is also quite variable from one embryo to another of the same age and even between litter mates. The total number of germ cells counted in 8-day embryos varied from 100 to 150, in 9-day embryos from 400–480, in 10-day embryos from 900–1000 and in 11-day embryos from 1575–1625. It was not found to be practicable to count the cells in the 12-day embryos since the cells were too densely packed within the germinal ridges and counts of the same series by two people varied more than 25%. The total number of germ cells in 12-day embryos is estimated to range between 2000 and 2500. There are no specific observations to indicate the mechanism of this increase in numbers, but presumably it is due to the mitotic activity of the migrating germ cells.

During the course of their migration, individual germ cells sometimes exhibit pseudopodia-like cell processes (fig. 5). These pseudopodia suggest the probable means of locomotion of the germ cells. The fixatives employed in this study are known to cause appreciable shrinkage and probably many of

the pseudopodia were retracted during fixation, thus explaining the infrequency of such cell processes.

DISCUSSION

The observations reported in this paper constitute the first complete description of the origin and migration of the primordial germ cells in the mouse embryo. Kirkham ('15) reported in an abstract the identification of germ cells in 11-day mouse embryos, but did not describe their distribution. Simkins ('23) made a careful study of rat and mouse embryos but found cells with the morphological characteristics of the germ cells so widely distributed that he concluded that the germ cells could not be identified by such criteria. Hargitt ('25) also was unable to identify germ cells in early rat embryos. More recently, Everett ('43) reported the identification of germ cells in the hind gut of a 2.6 mm deer mouse (Peromyscus maniculatus) embryo but could not recognize the cells in younger embryos. On the other hand, Everett presented very good experimental evidence for the existence of germ cells in the laboratory mouse and the present study provides the descriptive details to substantiate his conclusions.

The question of the origin of the germ cells must remain open. It would be precarious to argue that germ cells arise from only one specific site when the evidence to support such a conclusion is based solely on the study of fixed material. When the germ cells are first observed in the mouse embryo they are found in the yolk sac splanchnopleure, the caudal end of the primitive streak, and the root of the allantoic mesoderm. Which of these sites is the origin of the germ cells?

The fact that the germ cells in the primitive streak are surrounded by cells of varying enzymatic activity might suggest transition stages of somatic cells into germ cells. For this reason it was reported in the preliminary abstract of this study (Chiquoine, '53) that the germ cells probably arose from the primitive streak. Following this report I have studied

additional serial sections of 8-day embryos and I now no longer believe that the primitive streak is the primary origin of the germ cells.

In the youngest 8-day embryos, i.e., those having the fewest germ cells, the cells are most numerous in the splanchnopleure of the yolk sac. This observation leads me to believe that the germ cells in the mouse embryo arise from the yolk sac as has also been reported to be the case in man (Fuss, '11; Politzer, '33; Witschi, '48).

It is quite possible that the germ cells originate from both the yolk sac and the primitive streak. But even more important is the probability that the germ cells exist in earlier stages than observed in this study but that they do not possess the high enzymatic activity required for their demonstration. The tracing of the origin of the germ cells to the cleaving ovum is a much more fundamental problem.

Primordial germ cells which fail to attain the germinal ridges and which become lost in other tissues of the embryo have frequently been suspected of having a possible significance in the origin of embryomata. Witschi ('48) could not find any evidence from his study of the migration of germ cells in the human embryo to support this contention. Similarly, no evidence was found in the present study of the mouse embryo. Although a few germ cells were recognized in some embryos as having strayed from the germ-track, the location of these lost germ cells was never too far from the track. Embryomata, on the other hand, are known to occur in almost any part of the body and there are no observations to support such a widespread distribution of lost germ cells.

Witschi ('48) argued that the germ cells are guided in some way to the germinal ridges by chemotropism. My own observations on the infrequency of lost germ cells and the successful attainment of the germinal ridges by the vast majority of the germ cells are in agreement with this argument. Weiss and Andres ('52), from their study of the fate of embryonic cells injected into the circulatory system of chick embryos, suggest that the germ cells are distributed

at random throughout the embryo by the circulating blood and only those germ cells lodging in the germinal ridges survive and express their developmental potentialities. This may well be the case in the chick embryo, for germ cells have been observed in the blood stream (Willier, '50). In mammalian embryos, however, germ cells have not been reported in the circulating blood, nor have I ever observed them in the mouse embryo within the lumina of blood vessels. For these reasons, Witschi's suggestion of a positive chemotropism on the part of the germ cells is probably correct.

The significance of the high enzymatic activity of the germ cells is not known. The roles of alkaline phosphatase in embryonic tissues have been repeatedly discussed (Moog, '44; Brachet, '47; Chiquoine, '53), but the biochemical significance of this enzyme is so obscure that no definitive function can be assigned to it. It is quite apparent, however, that the ability of a cell to migrate is not dependent upon a high alkaline phosphatase activity since other migrating cells of the embryo do not possess such high levels of enzymatic activity, e.g., the cells of the neural crest during their migration to form the parenchyma of the adrenal medulla (unpublished observations).

SUMMARY

Employing the Gomori histochemical technique for alkaline phosphatase it has been possible to stain selectively the primordial germ cells of the mouse embryo. The germ cells were observed to originate from the yolk sac splanchnopleure and perhaps also from the caudal end of the primitive streak in 8-day embryos. The subsequent migration of the cells from their origin to the germinal ridges is described. In 12-day embryos, almost all the germ cells have attained the germinal ridge. Very few germ cells were observed to deviate from the germ-track and become lost.

These observations constitute the first complete description of the origin and migration of the primordial germ cells in the mouse embryo. This study also serves as a needed confirmation to other studies of germ cells in mammalian em-

bryos in which the identification of the cells has been based solely on morphological characteristics.

The significance of the high enzymatic activity of the germ cells is not known but it is not a characteristic of all migrating embryonic cells.

LITERATURE CITED

BRACHET, J. 1947 Nucleic acids in the cell and embryo. Symp. Soc. Exp. Biol., *1:* 207–224.

CHIQUOINE, A. D. 1953 The identification and migration of the primordial germ cells in the mouse embryo. (Abs.) Anat. Rec., *115:* 293.

———— Distribution of alkaline phosphomonoesterase in the central nervous system of the mouse embryo. J. Comp. Neur. (in press).

EVERETT, N. B. 1943 Observational and experimental evidences relating to the origin and differentiation of the definitive germ cells in mice. J. Exp. Zool., *92:* 49–91.

———— 1945 The present status of the germ-cell problem in vertebrates. Biol. Rev., *20:* 45–55.

FUSS, A. 1911 Über extraregionäre Geschlectzellen bei einem menschlichen Embryo von vier Wochen. Anat. Anz., *39:* 407–409.

———— 1912 Über die Geschlechtzellen des Menschen und der Saugetiere. Arch. f. mikr. Anat., *81*: 1–23.

GOMORI, G. 1946 The study of enzymes in tissue sections. Am. J. Clin. Path., *16:* 347–352.

HAMLETT, G. W. D. 1935 Primordial germ cells in a 4.5 mm human embryo. Anat. Rec., *61:* 273–279.

HARGITT, G. T. 1925 The formation of the sex glands and germ cells of mammals. 1. The origin of the germ cells in the albino rat. J. Morph. and Physiol., *40:* 517–557.

HEYS, F. 1931 The problem of the origin of germ cells. Quart. Rev. Biol., *6:* 1–45.

KIRKHAM, W. B. 1915 The germ cell cycle in the mouse. Anat. Rec., *10:* 217–219.

MOOG, F. 1944 Localization of alkaline and acid phosphatase in the early embryogenesis of the chick. Biol. Bull., *86:* 51–80.

NIEWKOOP, P. D. 1949 The present status of the problem of the "Keimbahn" in the Vertebrates. Experientia, *5:* 308–312.

POLITZER, G. 1933 Die Keimbahn des Menschen. Zeitsch. f. Anat. u. Entwicklungsgesch., *100:* 331–361.

RUBASCHKIN, W. 1908 Zur Frage von der Entstehung der Keimzellen bei Säugetierembryonen. Anat. Anz., *32:* 222–224.

———— 1909 Über die Urgeschlechtszellen bei Säugetieren. Anat. Anz., *39:* 603–652.

SIMKINS, C. S. 1923 Origin and migration of the so-called primordial germ cells in the mouse and rat. Acta Zool., *4:* 241–278.

———— 1928 Origin of sex cells in man. Am. J. Anat., *41:* 249–272.

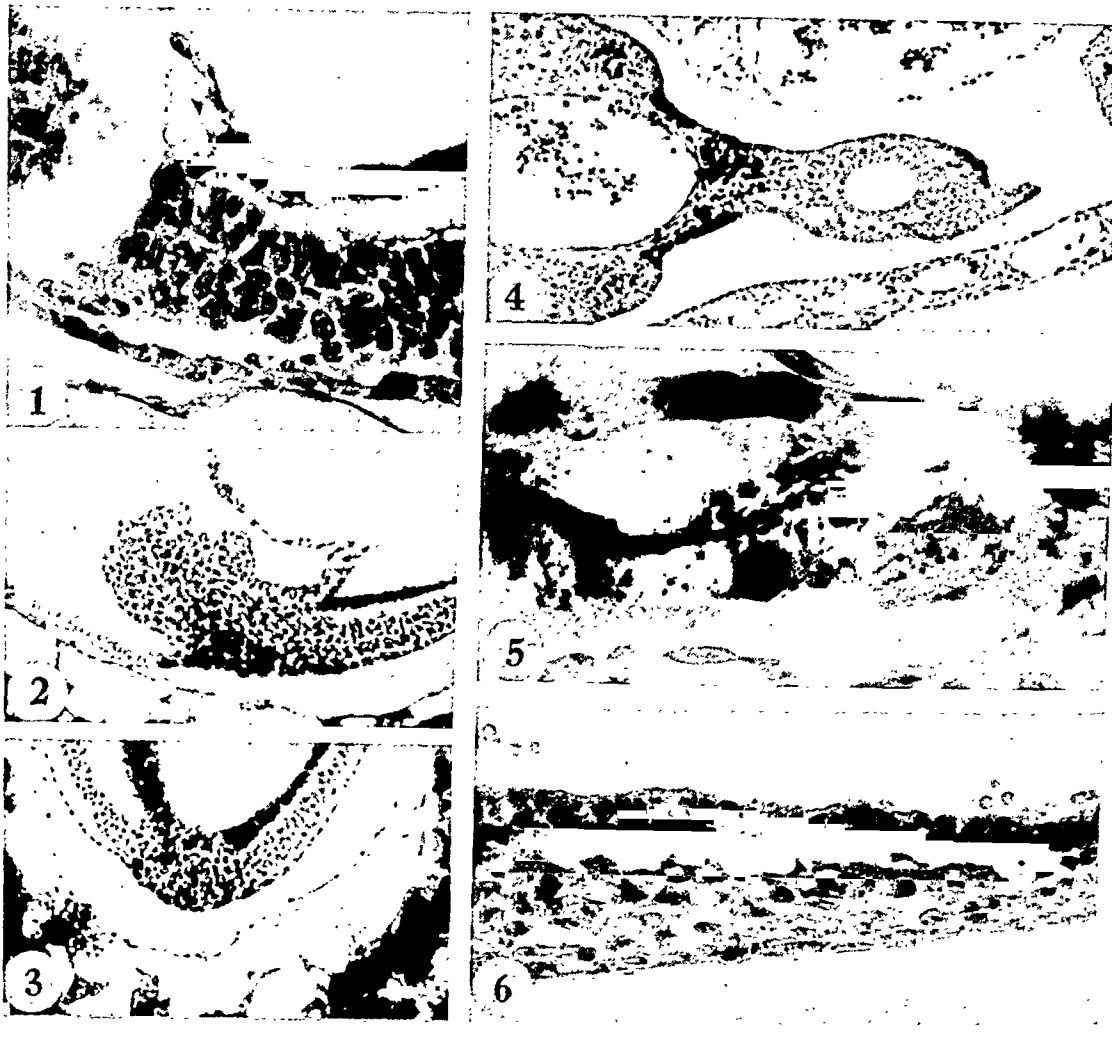
VANNEMAN, A. S. 1917 The early history of the germ cells in the armadillo, Tatusia novemcincta. Am. J. Anat., *22:* 341–364.

WEISS, P., AND G. ANDRES 1952 Experiments on the fate of embryonic cells (chick) disseminated by the vascular route. J. Exp. Zool., *121:* 449–488.

WILLIER, B. H. 1950 Sterile gonads and the problem of the origin of germ cells in the chick embryo. Arch. Anat. microscopique et Morph. exp., *39:* 269–273.

WITSCHI, E. 1948 Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Contr. Embryol. Carnegie Inst., *32:* 67–80.

WOLMAN, M., AND A. BEHAR 1952 A method of fixation for enzyme-cytochemistry and cytology. Exp. Cell Res., *3:* 619–621.



All the photographs on this plate are from serial sections of mouse embryos of known copulation age. The primordial germ cells are selectively stained black by virtue of their high alkaline phosphatase activity.

1 Sagittal section through the caudal end of the primitive streak of a young 8-day mouse embryo. Three primordial germ cells are selectively stained black in the entoderm of the yolk sac immediately beneath the primitive streak. × 340.

2 Sagittal section through the caudal end of the primitive streak and the allantoic mesoderm of a late 8-day mouse embryo. Numerous primordial germ cells are observed by virtue of their heavy staining in the yolk sac splanchnopleure and allantoic mesoderm. This section clearly indicates that the primordial germ cells originate from the yolk sac. × 100.

3 Cross section through the primitive streak of an 8-day embryo illustrating the darkly stained primordial germ cells in the entoderm and primitive streak. × 100.

4 Cross section of a 10-day mouse embryo. The primordial germ cells which are stained black can be identified in the root of the dorsal mesentery, within, and beneath the germinal epithelium. × 100.

5 Cross section of the hind gut of a 9-day embryo. The primordial germ cells are stained black and are present both in the entoderm and splanchnic mesoderm of the hind gut. The nuclei of the germ cells are essentially unstained but frequently appear black due to an overlying, stained cytoplasm. The germ cell situated just to the right of the middle of the photograph illustrates a pseudopodia-like process. × 340.

6 Frontal section of the germinal ridge of an 11-day embryo. The majority of the darkly stained primordial germ cells are situated immediately beneath the germinal epithelium. × 340.

THE STRUCTURE OF THE GOLGI APPARATUS

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THIRTEEN FIGURES

During the 55 years of its existence as an object of scientific inquiry, the Golgi apparatus has been interpreted in almost as many different ways as there have been techniques employed in its study. The importance ascribed to it has run the gamut from exaltation as the crucial center for secretory manufacture to dismissal as a technical nuisance or artifact. Rarely has the investigation of a natural phenomenon inspired such uncompromising partisanship, with preconceptions becoming tenets of faith instead of serving as working hypotheses. In no small part, this attitude has been fostered by the insecurity which is inherent when conclusions are drawn from methods whose basis is largely empirical. With refinements of cytochemical technique, the Golgi apparatus may be approached with greater assurance and allowed to record the responsiveness of its form and the character of its substance.

The Golgi apparatus cannot be merely an apparition, as frequently implied by reference to it as an artifact, since it has appeared to so many competent investigators in the same part of the cell after diverse experimental approaches. The one factor common to all of these experiments has been the presence of a cell, either alive or in some state of preservation. The Golgi images which have been observed must consequently owe their existence to the participation of some cellular component in the technical procedures. Whether this component changes greatly, or not at all, as it takes part in experiments, it is always Golgi material.

[1] With the technical assistance of Gloria Wayne.

Cellular constituents are routinely made presentable to the human eye by technical artifice without being stigmatized as artifacts. The image observed represents the living structure, modified by its response to the reagents employed. An embalmed geometrical replica of the original is not necessarily the most useful product. If a cell constituent, such as the Golgi material, responds to a chemical procedure by a recognizable change, the observed image records not only a modified form but a dynamic characteristic as well.

To expect the Golgi material to be so independent of its environment that it will not be sensibly altered by technical procedures is inconsistent with the view that it participates actively in the less drastic reactions characteristic of normal cell function. The Golgi material does respond to chemical procedures and the analysis of the pattern of the response enables us to acquaint ourselves with its dynamic characteristics. Although the expression of these characteristics in the living cell is the ultimate concern, their study can be approached most effectively through the accentuated reactions evoked by reagents which are not obliged to maintain a homeostatic environment.

The observations recorded in this paper will be restricted to the response of the Golgi apparatus of the Swiss albino mouse to selected reagents leading to paraffin embedding. The interpretation of these observations, however, has been guided by the results, as yet unpublished, of a great variety of chimical and physical experiments on supravital as well as fixed tissue.

Identification of the Golgi material

A common source of confusion in the discussion of the Golgi problem has been the lack of proper identification of the Golgi material. Too often identification has been allowed to rely on intuition, sharpened by experience, with the result that intuitions have become pointed in different directions. The formal definition of the Golgi apparatus as a cellular

entity is recorded in Golgi's original paper, published in 1898, although earlier cytological descriptions have since been interpreted as evincing awareness of its presence. In his paper, Golgi described the series of chemical reactions which he performed on the tissue and the appearance of the Golgi apparatus after these reactions. The primary criterion for the identification of Golgi material must consequently be its ability to respond to the technical procedures which Golgi employed so as to produce the structure which he obtained. It is obvious from this operational definition that structure alone is not sufficient for identification; the fundamental criterion is a chemical response, gauged by a physical effect.

The original technique employed by Golgi evolved by chance from his chrome-silver method for nerve. It was consequently more complicated than necessary and therefore capricious. Some simplification was achieved by Golgi ('08), Cajal ('12), Da Fano ('20), and Aoyama ('29) by the introduction of new fixatives and the employment of hydroquinone development. The elimination of previous fixation in the direct silver method of Elftman ('52) removed the fickleness characteristic of earlier methods, while retaining their diagnostic characteristics. Since each of these silver methods gives identical results, but the direct silver method is most consistent and simplest, it may now be regarded as the most convenient critical test for the presence of Golgi material.

The use of criteria other than its response to a properly executed silver technique for the identification of Golgi material lacks validity, unless the other methods have been carefully calibrated against the silver standard. The use of osmium tetroxide provides such a secondary criterion of identification, but only when used in certain authenticated procedures; haphazard osmication is not significant.

Characteristics of the silvered Golgi image

The fundamental pattern of the response of the Golgi material to the direct silver method can be studied in figures 2 through 5, showing tissue from the duodenum, pyloric stomach,

uterine gland and head of the epididymis. Fresh tissue was immersed directly in a 2% solution of silver nitrate in 5% formalin at pH 4.5 for two hours, followed by hydroquinone development. In all of these cells, the Golgi apparatus is supranuclear in position and can be recognized in the figures by the fact that it is more intensely silvered than are the surrounding structures. Identification as Golgi material, however, does not depend on position or mere attraction for silver but on the detailed structure of the silvered image.

To superficial observation, the Golgi apparatus in the 4 types of cells illustrated gives an impression of great variability, but closer study reveals the similarity of fundamental structure. In each may be recognized elongate elements, differing in size but not in intrinsic character. Even in thin sections, the tendency of these elements to terminate in bulbous enlargements is apparent, as is the fact that the interior of these enlargements has not reacted with the silver. When the Golgi material is more abundant and these elements are larger, as in the epididymis, the internal cavity, after direct silver treatment, is seen to be continuous within each element. The Golgi material consequently forms an interfacial film, separating the general cytoplasm from the contents of the internal space, referred to frequently as the Golgi internum.

Interpretation of the results of silver techniques on the Golgi apparatus must always be guided by consideration of the chemical reactions involved. In the first step of the direct silver method, the silver reacts with the Golgi material so as to produce a substance which serves as a nucleus for the deposition of metallic silver when hydroquinone is added, with uncombined silver still present in the tissue. The ensuing deposition of metallic silver is consequently similar to intensification as practiced in photography, and the size of the final silver deposit is determined by the degree of intensification and not by the thickness of the film of Golgi material. It is possible to carry the process so far as to fill all internal cavities with silver or to shorten it so that only the more reactive centers are intensified. Silver deposits

produced by this method are innately granular; there is no more reason for calling the granules mitochondria when they appear in a tissue section than when they occur on a photographic plate.

The more detailed characteristics of the silvered Golgi image have been considered first because they are the ones which are of diagnostic value. The Golgi elements which have been described may be grouped so as to give a superficial impression of forming a network or reticulum. This is more likely to occur when the thickness of the Golgi film has been greatly exaggerated by excessive silvering, so that elements which are actually discrete appear to coalesce. The interstices of such a network, as seen in plane section, are partially Golgi internum and partially cytoplasmic space.

Effect of variations in silver technique

In order to test whether the configuration of the Golgi apparatus has been influenced by the reagents employed in the direct silver method, it is possible to repeat the procedure with controlled variations in the reagents. The results of such experiments are shown in figures 6 through 8. The epididymis has been chosen as the test object, since it is generously provided with Golgi material. The marked variations in the character of the cells in different regions of the epididymis, especially in its head, requires that great care be exercised in choosing similar cells for comparison. This can be accomplished easily in tissue sections but presents a serious problem in other methods of study.

When the silver reaction is conducted at pH 7.2 with the formalin concentration reduced to 1%, the Golgi material responds by producing the structure shown in figure 6. The silvered image is smaller than that produced by the direct silver method, figure 5, but still gives evidence of organization into a film surrounding an unreactive interior. When the formalin is eliminated, but the pH kept at 7.2, the Golgi apparatus presents itself in a condensed, globular form, as in figure 7.

Further evidence of the powerful effect which the chemical treatment has on the structure of the Golgi apparatus is provided by figure 8. After subjection to 1.5% ammoniacal silver nitrate, pH 10.3, for two hours, the tissue was developed for one hour in 2% hydroquinone dissolved in 15% formalin. The Golgi apparatus has responded by extreme expansion.

Effect of osmium

For many decades the use of osmium mixtures for the demonstration of the Golgi apparatus has enjoyed greater popularity than has the use of silver, largely due to the difficulties encountered in the application of the earlier silver methods. Typical of the results achieved with osmium is figure 9 of the epididymis, prepared by the method of Ludford ('26). The major characteristics produced by this method correspond closely with those obtained by the direct silver procedure, figure 5. In both cases the thickness of the interfacial membrane is exaggerated by excessive secondary deposition.

Having demonstrated that the Golgi apparatus is expanded by the formalin component of the direct silver method, the suspicion is naturally aroused that some component of the osmium method has performed the same service. That osmium can be applied in such a fashion as to circumvent expansion is demonstrated by figure 10. This tissue was immersed in 2% osmium tetroxide, buffered according to the recommendations of Palade ('52), for three hours at 37°. Under these conditions osmium does not blacken the Golgi material but presumably forms an addition product of the general type investigated by Criegee ('36, '42). Reduction of the osmium attached to the Golgi apparatus, without general blackening of the tissue, was accomplished by the subsequent action of 1% formalin in the presence of 1% potassium dichromate and 2% osmium tetroxide, overnight at 56°, an initial pH of 3.5 being provided by acetate buffer. Under these circumstances the Golgi apparatus of the epi-

didymis emerges more condensed than it is after prolonged osmication preceded by the customary fixatives. It resembles more closely the results achieved with neutral silver in the absence of formalin.

Effect of dichromate

Dichromate has been used as an ancillary reagent in Golgi procedures from Golgi's earliest method to the present time. When 2.5% potassium dichromate is dissolved in 20% formalin to form Regaud's fixative, overnight fixation of the epididymis at 37° results in the Golgi configuration shown in figure 11. The use of fast green as a background stain allows the shape of the Golgi film to be appreciated by contrast, since it encloses the unstained Golgi internum. This is an example of what is usually referred to as a negative Golgi image, a designation which would be more accurate if the Golgi internum were not customarily refractory to staining. In any case, the organization of the Golgi film in tubules with dilatations may be compared with its response to silver and osmium.

In contrast to its employment as an accessory reagent, utilization of dichromate as a primary reactant for the Golgi apparatus dates from Baker's application of the Smith-Dietrich method in 1944. By this means Baker made the important contribution of substantiating the presence of phospholipids in the Golgi material. A further refinement of this general method, using controlled chromation, has been achieved during the present study. The essential feature of this method is the control of pH as well as temperature, concentration, and time.

One example of the response of the Golgi material of the duodenum to controlled chromation is presented in figure 12. After reacting with 2.5% potassium dichromate at pH 3.5 for 18 hours at 56°, the Golgi material has been sufficiently oxidized to survive embedding in paraffin. It may then be stained with a fat-soluble dye, such as the Sudan black B

used for figure 12, or the trivalent chromium resulting from the reaction may be stained with acid hematein.

In contrast to figure 12, in which formalin has not been used, is figure 13, fixed overnight at room temperature in Orth's fluid, which contains 2.5% potassium dichromate and 10% formalin. The less complete preservation of the Golgi material, compared to figure 12, is attributable to the higher pH and lower temperature, while the different expansion of the Golgi apparatus is due to the addition of formalin to the dichromate.

Interpretation of observations

Although it would be possible to extend the line of evidence put on record here by describing the effect of other reagents on the Golgi material, the experiments described are sufficient to substantiate the conclusion that the Golgi material is responsive to changes in its chemical environment. The pattern of these responses is determined by characteristic properties of the Golgi material.

The structures assumed by the Golgi apparatus after the various reactions represented by figures 2 through 13 range in a continuous series from a condensed globule without visible internal cavity to greatly expanded configurations with the Golgi material forming an interfacial film between a central fluid and the surrounding cytoplasm. Moderately expanded stages retain an essentially spherical outline, while further expansion results in elongation. With this elongation, a characteristic tubular shape with bulbous terminations is assumed when the pressure of the surrounding cell constituents does not interfere. The terminal dilatations have a strong tendency to exhibit a diameter twice that of the tubular connecting portion. The mean curvature of these two portions of the figure are consequently the same, a strong indication that the shape is related to the tension of the film of Golgi material.

When the Golgi material is in the expanded state, the area of the interfacial film which it forms can be determined. One

method of doing this is illustrated by the nomograph of figure 1. The two scales to the right refer to dimensions of the expanded Golgi apparatus in microns, when it assumes the form of a tube with hemispherical ends of the same diameter

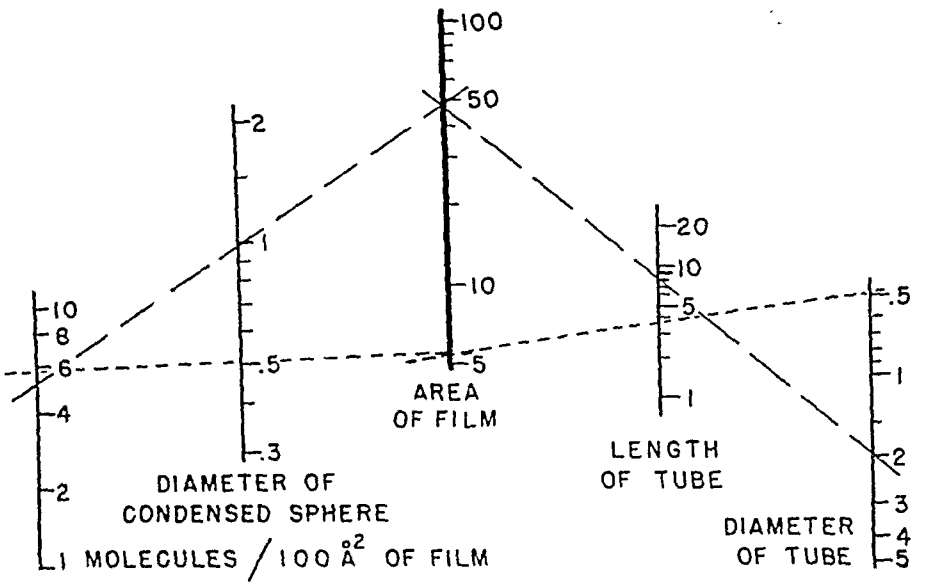


Fig. 1 Nomograph of some of the physical properties of the Golgi apparatus. The central scale gives the area of the film of Golgi material when it is expanded in the form of a hollow tube, the length and diameter of which are shown on the two scales at the right, in microns. The scales at the left give the concentration of Golgi material in the film and the diameter of the sphere into which this material would condense with a density of 0.9 and a molecular weight of 800. The broken and dotted lines represent preliminary values for the particular part of the epididymis used in the present study and for the absorptive cells of the duodenum. The observed values used in locating these lines were the length and diameter of the Golgi apparatus when expanded by the direct silver and Ludford osmium techniques and the diameter of the Golgi globules observed after minimal expansion.

as the tube. This reference shape is convenient for computation because it becomes a sphere when the length and diameter are equal and the increase in area provided by terminal dilatations can be adjusted for by added reference tube length. A line drawn through the values for length and diameter of the tube intersects the central scale to give the area of the film in square microns.

Two other properties of the film are indicated by the scales on the left half of the nomograph. The first is the concentration of Golgi material in the film, expressed in terms of the number of molecules for each 100 square Ångstroms of area. The second is a measure of the total amount of Golgi material present in a given area of film. If all of the material present in a given area at a given concentration were condensed into a sphere, the diameter of the sphere would furnish a convenient index of its volume. This diameter will depend on the molecular characteristics of the material involved; the scale on the nomograph is constructed for a density of 0.9 and a molecular weight of 800, adjustment for other values being a simple matter.

The relations used in the construction of the nomograph are purely mathematical and are consequently independent of any particular interpretation of the Golgi apparatus. When measurements of actual preparations are plotted on the nomograph, interesting correlations appear. Two such sets of preliminary data have been plotted in figure 1. The dotted lines represent the Golgi apparatus of the absorptive cells of the duodenum, and the broken lines the particular portion of the epididymis used in the present study. Starting with measurements of the length and diameter of the expanded Golgi apparatus of direct silver and Ludford osmic preparations, the area of the Golgi film is found. When a line is drawn from this value for the area through the observed value for the diameter of the condensed Golgi apparatus produced by other techniques, the scale at the left is intersected in the general region of 5 or 6 molecules for each 100 square Ångstroms of film area.

In view of the preliminary nature of the data employed, these particular numerical values for concentration of Golgi material in the film should not receive undue emphasis, but the general order of magnitude which they indicate is reliable. When these results are studied in conjunction with the data on interfacial phospholipid films, most easily accessible in Adam ('41), it is apparent that the production of the ex-

panded Golgi forms from the condensed spheres is well within the power of the material which is predominant in them.

DISCUSSION

The Golgi material is a dynamic constituent of the cell which reveals some of its fundamental characteristics by the response which it gives to changes in its chemical environment. Since the amount of Golgi material, and the details of its chemical composition, vary from cell to cell, the responses vary in detail but follow a general pattern. The key to this pattern is the ability of the Golgi material to form interfacial films of different degrees of expansion under different chemical circumstances. The form which it will assume in a particular living cell can therefore by expected to depend on the particular variety of Golgi material present in that cell and the particular chemical environment surrounding it at the moment.

Recognition of this property of the Golgi material allows the observation of the varieties of Golgi apparatus in living cells to proceed with greater objectivity. The question is not whether the Golgi apparatus is always tubular or always globular but what degree of condensation or expansion is characteristic of it in each cell type under specific physiological conditions. Extension and refinement of interpretation of the types of observation made by Bensley ('11), O'Leary ('30), Palade and Claude ('49), Baker ('49), Thomas ('48, '49), Xeros ('51), Dalton ('53), and Adamstone and Taylor ('53) will eventually allow the spectrum of living Golgi structures to be classified in accordance with their position in the condensation-expansion series. The sensitivity of the Golgi material to changes in its environment, and the exceptional hazards involved in proper identification, necessitate extreme care in evaluating the reports of direct observation.

One of the striking features of the experiments reported in this paper is the tendency of the Golgi material to respond to the less drastic reagents by appearing in a condensed,

globular form. This is in accord with the experiences of Palade and Claude ('49) with homogenates. There is consequently good reason for believing that the spectrum of living Golgi structures will eventually be found to be heavily skewed in favor of condensed, globular forms. Certainly globular lipid "inclusions" merit the closest scrutiny from those who are searching the cell for normal Golgi material. The extent to which the coalescence of droplets, according to the concept of Worley ('46, '51), contributes to the formation of observed Golgi bodies requires further investigation.

The significance of the Golgi internum, the contents of the space enveloped by the expanded Golgi film, is still subject to question. Since the volume of this space varies with changes in the chemical environment, the first assumption would be that its contents are derived from the surrounding cytoplasm by filtration through the semipermeable membrane provided by the Golgi film. Analysis of the Golgi internum should yield valuable information concerning the permeability of the Golgi material.

The debt which anyone working with the Golgi apparatus at the present time owes to his countless predecessors is apparent. The literature which has accumulated in such prodigious fashion has fortunately been expertly reviewed by Kirkman and Severinghaus ('38), Hirsch ('39), Hibbard ('45), and Bensley ('51). In the course of its long history, the genesis of basic concepts concerning the Golgi apparatus has become veiled by the passage of time, and the only sure way to honor all who have given birth to good ideas is to nurture them to maturity. It is possible, however, to give specific credit to 5 investigators who have made conspicuous contributions to the basic concepts elaborated in the present paper. Virchow (1854) alerted microscopic anatomy to the physical transformations manifested by myelin-like substances. Sjövall ('06) pioneered the study of the response of the Golgi material to chemical reagents and its interpretation in terms of myelin-like constituents. Baker ('44) applied cytochemical methods to the Golgi material and proved

the presence of phospholipids. Palade and Claude ('49) brought into modern focus the relation between the physical transformations of the Golgi material and its lipid component.

SUMMARY OF CONCLUSIONS

The Golgi material is a real constituent of the cell and participates in experiments performed upon it. The response which it gives to silver techniques provides a definitive means of identification which has historical validity. One fundamental property of the Golgi material is its ability to undergo different degrees of expansion into interfacial films in response to various reagents. This sensitivity to the environment affords the possibility that the living Golgi materials of different cells, differing as they do in details of chemical constitution and in amount, may exhibit different degrees of expansion. They may then be classified according to their position on the condensation-expansion scale. Condensed Golgi globules in living cells deserve especial attention; their identification cannot be achieved by direct observation but may be obtained by subsequent application of the direct silver reaction. The responsiveness of the Golgi material to its chemical environment is indicative of its dynamic character and is consonant with its phospholipid content.

LITERATURE CITED

ADAM, N. K. 1941 The physics and chemistry of surfaces. Oxford.

ADAMSTONE, F. B., AND A. B. TAYLOR 1953 Structure and physical nature of the cytoplasm of living spinal ganglion cells of the adult rat. J. Morph., *92:* 513–539.

AOYAMA, F. 1929 Eine Modification der Cajalschen Methode zur Darstellung der Golgischen Binnennetz Apparate. Zeit. wiss. Mikr., *46:* 489–491.

BAKER, J. R. 1944 The structure and chemical composition of the Golgi element. Quart. J. Mic. Sci., *85:* 1–71.

——— 1949 Further remarks on the Golgi element. Quart. J. Mic. Sci., *90:* 293–307.

BENSLEY, R. R. 1911 Studies on the pancreas of the guinea pig. Am. J. Anat., *12:* 297–388.

——— 1951 Facts versus artefacts in cytology: the Golgi apparatus. Exp. Cell Research, *2:* 1–9.

CAJAL, S. RAMON Y 1912 Formula de fijacion para la demonstracion fácil del aparato reticular de Golgi. Trab. Labor. Invest. Biol. Univ. Madrid, *10:* 209–220.

CRIEGEE, R. 1936 Osmiumsäure-ester als Zwischenprodukte bei Oxydationen. Liebig's Annalen d. Chemie, *522:* 75–96.

CRIEGEE, R., B. MARCHAND AND H. WANNOWIUS 1942 Zur Kentniss der organischen Osmium-Verbindungen. Liebig's Annalen d. Chemie, *550:* 99–133.

DA FANO, C. 1920 Method for the demonstration of Golgi's internal apparatus. J. Physiol., *53:* xcii–xciv.

DALTON, A. J., AND M. D. FELIX 1953 Studies on the Golgi substance of the epithelial cells of the epididymis and duodenum of the mouse. Am. J. Anat., *92:* 277–305.

ELFTMAN, H. 1952 A direct silver method for the Golgi apparatus. Stain Tech., *27:* 47–52.

GOLGI, C. 1898 Sur la structure des cellules nerveuses. Arch. Ital. Biol., *30:* 60–71.

———— 1908 Une méthode pour la prompte et facile démonstration de l'appareil rèticulaire interne des cellules nerveuses. Arch. Ital. Biol., *49:* 269–274.

HIBBARD, H. 1945 Current status of our knowledge of the Golgi apparatus in the animal cell. Quart. Rev. Biol., *20:* 1–19.

HIRSCH, G. C. 1939 Form und Stoffwechsel der Golgi-Körper. Protoplasma Monographien, vol. 18.

KIRKMAN, H., AND A. E. SEVERINGHAUS 1938 A review of the Golgi apparatus. Anat. Rec., *70:* 413–431, 557–573 and *71:* 79–103.

LUDFORD, R. J. 1926 Further modifications of the osmic acid methods in cytological technique. J. Roy. Micr. Soc., *46:* 107–109.

O'LEARY, J. L. 1930 An experimental study of the islet cells of the pancreas in vivo. Anat. Rec., *45:* 27–58.

PALADE, G. E. 1952 A study of fixation for electron microscopy. J. Exp. Med., *95:* 285–298.

PALADE, G. E., AND A. CLAUDE 1949 The nature of the Golgi apparatus. J. Morph., *85:* 35–112.

SJÖVALL, E. 1906 Über Spinalganglionzellen und Markscheiden, zugleich ein Versuch die Wirkungsweise der Osmiumsäure zu analysieren. Anat. Hefte, *30:* 259–391.

THOMAS, O. L. 1948 A study of the spheroid system of sympathetic neurones with special reference to the problem of neurosecretion. Quart. J. Mic. Sci., *89:* 333–350.

———— 1949 A comparative study of the cytology of the nerve cell with reference to the problem of neurosecretion. J. Comp. Neurol., *95:* 73–99.

VIRCHOW, R. 1854 Über das ausgebreitete Vorkommen einer dem Nervenmark analogen Substanz in den thierischen Geweben. Virchow's Arch. f. path. Anat., *6:* 562–572.

WORLEY, L. G. 1946 The Golgi apparatus, an interpretation of its structure and significance. N. Y. Acad. Sci. Annals, *47:* 1–56.

WORLEY, L. G. 1951 Recovery of the Golgi apparatus from homogenates of normal mammalian liver. Exp. Cell Research, *2:* 684–687.

XEROS, N. 1951 Lipoid bodies, Golgi apparatus and zymogen formation. Nature, *167:* 448–449.

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs, × 800, of paraffin embedded tissues of the Swiss albino mouse. The sections are 5 μ thick, except for figures 2, 3, 4, and 12, which were cut at 2 μ.

2–5 Golgi apparatus of the duodenum, pyloric stomach, uterine gland and one type of epididymal tubule after the direct silver method, using 2% silver nitrate in 5% formalin, pH 4.5, for two hours, followed by hydroquinone development. The differences shown by these tissues are partly due to the different amounts of Golgi material, partly to chemical differences in constitution. The common pattern of response includes elongate elements with bulbous hollow terminations, the Golgi material being represented by a film of silver forming an interface between the interior fluid and the surrounding cytoplasm. The thickness of the film is exaggerated by the deposition of excess silver.

6–7 Change in the response of the Golgi apparatus of the epididymis to silver when the pH is raised to 7.2 and the formalin concentration is reduced to 1% in figure 6 and formalin is eliminated in figure 7.

8 Expanded Golgi apparatus of the epididymis produced by 1.5% ammoniacal silver nitrate, pH 10.3, for two hours, followed by hydroquinone-formalin development.

9–10 Effect of osmium tetroxide on the Golgi apparatus of the epididymis when applied by Ludford's method, figure 9, and by fixation in Palade's buffered osmium followed by dichromate-formalin development, figure 10.

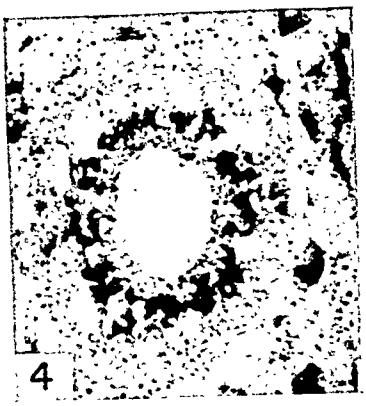
11 Epididymis fixed in 2.5% potassium dichromate, 20% formalin (Regaud) at 37° overnight, with fast green used as a background stain. The "negative" image of the Golgi apparatus shows characteristic tubular development with bulbous terminal expansions.

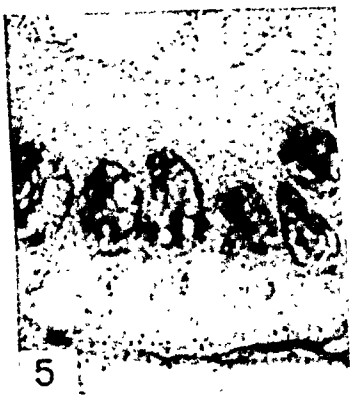
12 Duodenum prepared by the controlled chromatin method, with 2.5% potassium dichromate, pH 3.5, at 56° for 18 hours. Stained with Sudan black B. The phospholipid component of the Golgi apparatus is visualized by this method.

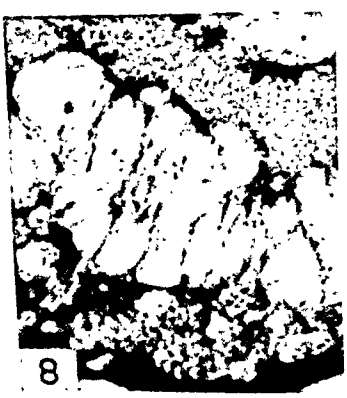
13 Duodenum after fixation in 2.5% potassium dichromate, 10% formalin (Orth), and stained with Sudan black B. The Golgi apparatus is less completely preserved than in figure 12 but it shows the greater expansion due to formalin.

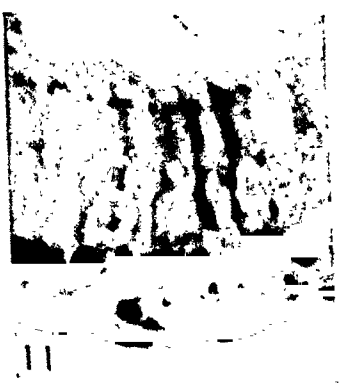
STRUCTURE OF GOLGI APPARATUS

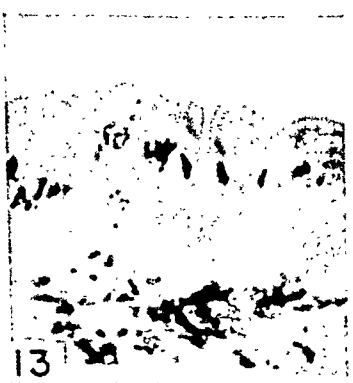
HERBERT ELFTMAN











AGENESIS OF THE EXTERNAL GENITALIA WITH REPORT OF TWO CASES

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FOUR FIGURES

Total agenesis of the external genitalia is a very rare anomaly. Only 5 cases were found recorded in the literature (Martin, 1878; Willett, 1894; Beaumont, '03a, '03b; Choisy, '26; and Kirshbaum, '50). Absence of parts of the external female genitalia in two sisters was reported by Mabaret du Basty (1890). In sireniform monsters the external genitalia are usually absent since they are included in the deficient caudal end of the trunk, as reported by Heury (1894), Manners-Smith (1896), Gladstone ('06), Johnston ('20), and Dawson ('22a).

MATERIAL

The pelvic and abdominal organs of two human female specimens form the basis of the present study.

Case 1. One was an anencephalic fetus about 8 lunar months of age, with no case history available. The crown-rump length of the specimen was 17 cm. It was designated Fetus A in an earlier paper (Warren, '51).

Case 2. The second specimen was a new-born baby that had died 27 hours post-partum. The mother, 34 years of age, had borne three normal children. She was treated by cervical cautery up to the third month of pregnancy. Gestation period 9 lunar months plus 10 days. Additional anomalies observed in this specimen were: gastroschisis (X in fig. 4), ectopia vesicae, right talipes calcaneovarus (T in fig. 4), a deficient left inferior extremity (F in fig. 4) without a foot,

and a large cyst in the left gluteal region. Histological sections of some of the internal genital organs were stained with hematoxylin and eosin.

OBSERVATIONS

Case 1. Externally the perineum presented a small genital tubercle at the anterior end of a low median raphé, shown at R in figure 1. No orifice whatever could be found in the perineum. The nates and inferior extremities were formed perfectly. A sacral dimple was present (S in fig. 1).

Internally paired ovaries with ova (RO, LO in fig. 2), paired uterine tubes (RT, LT in fig. 2), and a bicornuate uterus (UB, fig. 2) occupied the usual locations in the broad ligaments. The intestines were contained in the sac of a dorsal lumbar hernia illustrated previously (figs. 8–9, Warren, '51). A left diaphragmatic hernia permitted the stomach, left adrenal gland and left kidney to pass into the cavity of the thorax, while the pyloric junction and left ureter remained in the hiatus (D and LU in fig. 2). The right kidney was large, almost circular in shape (RK in fig. 2), and the right ureter was also enlarged. The left kidney was smaller and its ureter thinner. The adrenal glands appeared to be relatively large, but were normal histologically.

The right hypogastric artery was large in diameter and was continued as a solitary umbilical artery, similar to that described by Dawson ('22b). The left umbilical artery was absent, but there was a very thin aberrant artery (A in fig. 2), which arose from the abdominal aorta near the level of the renal arteries, passed across the peritoneal cavity to the base of the umbilical cord, and joined the solitary (right) umbilical artery.

Case 2. Exstrophy of the urinary bladder with gastroschisis and a rectovesical fistula caused a very peculiar perineum devoid of any orifices. In this specimen a very low raphé was present immediately to the right of the mid-sagittal plane, but with no other sign of the external female genitalia. Ex-

ternally this case presented a very thin anterior abdominal wall (X in fig. 4), a malformed left hip with a large cyst and a defective left inferior extremity (F in fig. 4), and a right talipes calcaneovarus (T in fig. 4). Due to the gastroschisis, the colon (C in fig. 4) presented itself on the anterior surface of the specimen.

Internally paired ovaries with ova and paired uterine tubes with fimbria were located immediately caudal to the diaphragm in the upper right and upper left quadrants of the posterior abdominal cavity, as shown in figure 3 (RO, LO, RT, LT). The left uterine tube terminated blindly in the retroperitoneal fascia about 15 mm from its fimbria (LT in fig. 3), with no connection to the uterus. The right uterine tube was about 20 mm in length, and was connected to a small cylindrical unicorn uterus (UU in fig. 3). The latter measured 10 mm in length and had a diameter of 4 mm. It is interesting to note that Beigel (1877) described a larger right unicorn uterus 30 mm in length. A broad ligament was not developed, so the small unicorn uterus was located on the right side of the abdomen, dorsal to the right border of the urinary bladder (V in fig. 3) and adherent to the latter. The uterus was without a cervix and the vagina was absent.

Parallel to the right uterine tube was another tube that appeared histologically to be a ductus deferens, or Wolffian duct. There was neither a testis nor an ovotestis, only the paired ovaries which were normal histologically.

The left kidney was ovoid in shape, had two long longitudinal sulci, and lacked a hilum. The ureter arose from the caudal border of the kidney, passed along the dorsal wall of the abdomen and opened on the everted inner surface of the urinary bladder. The right kidney was smaller than the left but similar to it in shape.

The gastrointestinal tract was normal grossly except for the stenotic rectum (SR in fig. 3). The rectum opened by a small orifice on the everted inner surface of the urinary bladder. Both adrenal glands were large, and seemed normal histologically.

DISCUSSION

The fact that each fetus was a female suggests that agenesis of the external genitalia, at least in these cases, might be due to the fusion of the labia majora into a raphé combined with rectal atresia. The median raphé present in each specimen resembled the perineal, or scrotal raphé of the normal male, except that the fusion had continued anteriorly to include the genital tubercle, and thus obliterated all the usual orifices. That this is possible, although rare, is suggested by the cases of Harris and Barry (1847) and Haskins (1851). Harris' case was an 18 year old slave, who menstruated regularly through the "penis," which was an inch long. The anus was patent, but there was no other orifice. Haskins described a slave 21 years old, of general female appearance, but with a "penis" an inch and a half long. The glans penis had a fissure instead of an urinary meatus, and says Haskins: "The fissure terminated by the apparent union of its lips, forming a cicatrix, which resembled somewhat the raphé of a scrotum. This raphé extended to what should have been the position of the posterior commissure of the vulva in the female."

Another explanation should be mentioned, although it seems to me to be less probable. It does not account for the median raphé present in Haskins' and the author's cases. It is possible that a premature growth, or an overgrowth, of the urorectal fold fused with a persistent cloacal membrane near the base of the tailbud, and thus separated the rectum from the surface. Consequently the proctodeum would not be stimulated to develop and anal atresia resulted.

In each of the author's cases there was a defect in at least one of the Müllerian ducts. In case 2 the failure of the left uterine tube to unite with the uterus is a condition more frequent than is generally realized. In this case the urinary system was not affected, therefore the developmental arrest must have occurred relatively late. Also the distance between the uterine tube and the urinary bladder was much greater on the left than on the right side. Brackenbury (1891) and Schattenberg and Ziskind ('40) have reported such cases.

Dannreuther ('23) found a unicorn uterus in a married woman 25 years old, and Mishell ('38) found agenesis of the right ovary as well as a mere "stump" of the uterine tube on the same side in an adult woman. For a complete review of the pertinent literature the reader is referred to Shumacker ('38). The bicornuate uterus of case 1 suggests that a developmental failure of fusion occurred only in the caudal portion of the Müllerian ducts, although both ducts must have been fully formed.

Exstrophy of the urinary bladder in case 2 was probably related to the gastroschisis and followed failure of the urogenital sinus to develop normally (Patten and Barry, '52). Connell ('01), Johnston ('14), Wyburn ('37), Goell ('50), and Harlin and Fort ('51), among others, have reviewed the literature, described similar cases, and given plausible explanations of this very unfortunate anomaly which appears to be confined to humans.

A large literature has accumulated on the subjects of anal atresia and rectal stenosis. Ladd and Gross ('34) reviewed 162 cases and Lu ('50) 74 cases. Rectovesical fistula has been described by Brenner ('15) and others. It is frequently associated with anal atresia.

Solitary umbilical artery has been described by Dawson ('22b) and its various sites of origin were classified. Wolff (1898–1899) has reviewed the literature on this interesting subject. However, the author has found no description of an aberrant artery to the umbilicus. It is difficult to envision the process by which any of the normal embryonic arteries could persist to form the above mentioned aberrant umbilical artery.

SUMMARY

Total agenesis of the external genitalia is described in two female human fetuses. The external genitalia were represented only by a low mid-sagittal ridge in the perineum; which, in one case, ended in a genital tubercle. One fetus possessed a bicornuate, and the other a unicorn uterus. Paired ovaries with ova and paired uterine tubes were present in both.

Additional multiple anomalies were present in each case, but of these only anal atresia was common to both.

LITERATURE CITED

BEAUMONT, E. 1903a Absence of sexual organs. Brit. Med. J., *1903* (II): 1152.

———— 1903b Case of a child with apparently complete absence of sexual organs. Trans. Clin. Soc. of London, *37:* 216.

BEIGEL, H. 1877 Uterus unicornis dexter. Arch. f. Gyn., *11* (2): 380.

BRACKENBURY, H. B. 1891 Suppression of urine in a case of single kidney, with an unusual malformation of the generative organs. Lancet, *1891* (II): 869–870.

BRENNER, E. C. 1915 Congenital defects of the anus and rectum. Surg., Gyn. and Obs., *20* (5): 579–588.

CHOISY, R. 1926 Sur l'absence des organes génitaux externes et la peritonite externe. Gynéc. et Obst., Paris, *13* (3): 177–204.

CONNELL, F. G. 1901 Exstrophy of the bladder. J. Am. Med. Assn., *36* (10): 637–668.

DANNREUTHER, W. T. 1923 Dextroversion of the uterus, with congenital absence of the left fallopian tube, ovary, broad ligament, round ligament, kidney and ureter. Am. J. Obst. and Gyn., *6* (1): 51–53.

DAWSON, A. B. 1922a Some significant features of the anatomy of a symelian monster (Sympus monopus). Anat. Rec., *23* (1): 15.

———— 1922b The origin and occurrence of the single umbilical artery in normal and abnormal human fetuses. Anat. Rec., *24:* 321–343.

GLADSTONE, R. J. 1906 A symelian monster (Sympus dipus). Brit. Med. J., *1906* (II): 1704.

GOELL, E. M. 1950 Über eine komplizierte Missbildung am kaudalen Rumpfende. Zentralb. f. allg. Path. u. path. Anat., *86* (5–6): 276–281.

HARLIN, H. C., AND C. A. FORT 1951 Exstrophy of the urinary bladder. Brooklyn Hosp. J., *9* (3): 132–144.

HARRIS, S. H., AND W. J. BARRY 1847 A case of doubtful sex. Am. J. Med. Sci., *14* (no. 7): 121–124.

HASKINS, W. D. 1851 Report of a case of doubtful sex. Boston Med. and Surg. J., *44:* 57–59.

HEURY, J. P. 1894 A sexless monster. Brit. Med. J., *1894* (I): 1343.

JOHNSTON, T. B. 1914 Extroversion of the bladder, complicated by the presence of intestinal openings on the surface of the extroverted area. J. Anat. and Phys., *48* (1): 89–106.

———— 1920 The anatomy of a symelian monster. J. of Anat., London, *54:* 208–216.

KIRSHBAUM, J. D. 1950 Congenital absence of the external genitals; persistent primitive cloaca. J. of Pediat., St. Louis, *37* (1): 102–105.

LADD, W. E., AND R. E. GROSS 1934 Congenital malformations of anus and rectum. Report of 162 cases. Am. J. of Surg., n.s., *23* (1): 167–183.

LU, W. S. 1950 Malformations of the anus and rectum; an analysis of 74 cases. Chinese Med. J., Shanghai, *68* (5–6): 123–138.

MABARET DU BASTY 1890 Absence d'une partie des organes génitaux externes chez deux soeurs. Le Prog. Méd., *1890–1891:* 503.

MANNERS-SMITH, T. 1896 A description of two symelian monsters. J. Anat. and Phys., *30* (2): 169–184.

MARTIN, E. 1878 Mémoire sur un cas de persistence des canaux de Müller. Oblitération des voies urinaires. Neutralité sexuelle. J. d'Anat. et de Phys., *14:* 21–33.

MISHELL, D. R. 1938 Unilateral absence of the fallopian tube and ovary. Am. J. Obs. and Gyn., *36* (4): 705–706.

PATTEN, B. M., AND A. BARRY 1952 The genesis of exstrophy of the bladder and epispadias. Am. J. Anat., *90* (1): 35–57.

SCHATTENBERG, H. J., AND O. J. ZISKIND 1940 Right uterus unicornis associated with renal agenesis. Am. J. Obs. and Gyn., *40* (2): 293–296.

SHUMACKER, H. B. 1938 Congenital anomalies of the genitalia associated with unilateral renal agenesis. Arch. of Surg., *37:* 586–602.

WARREN, H. S. 1951 Acrania induced by anencephaly. Anat. Rec., *111* (4): 653–668.

WILLETT, G. 1894 A sexless monster. Brit. Med. J., *1894* (I): 1168.

WOLFF, B. 1898-99 Ueber Missbildungen mit einfacher Nabelarterie. Arch. f. Gyn., *57:* 635–661.

WYBURN, G. M. 1937 The development of the infra-umbilical portion of the abdominal wall, with remarks on the aetiology of ectopia vesicae. J. of Anat., London, *71* (2): 201–231.

ABBREVIATIONS

A., arteria aberans
B., border of left diaphragmatic hernia
C., colon
D., duodenum
E., esophageo-cardiac junction
F., femur of defective left inferior extremity
G., genital tubercle
L.A., left umbilical artery
L.O., left ovary
L.T., left uterine tube
L.U., left ureter
R., ridge in midline of perineum
R.A., right umbilical artery
R.K., right kidney
R.O., right ovary
R.T., right uterine tube
S., sacral dimple
S.R., stenotic rectum
T., talipes calcaneovarus
U., umbilical cord
U.B., uterus bicornis
U.U., uterus unicornis
V., urinary bladder
X., gastroschisis

PLATE 1

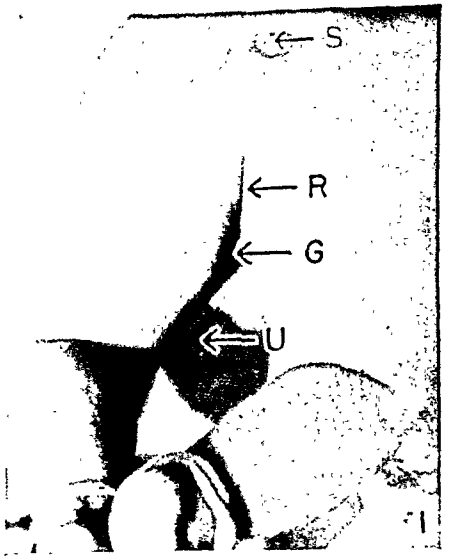
EXPLANATION OF FIGURES

1 Inferior aspect of case 1, showing the perineum with a genital tubercle and mid-sagittal ridge, or raphé, without any orifices. × ½.

2 Case 1, anterior aspect of dissected abdominal and pelvic cavities, showing the internal female genital organs, the aberrant umbilical artery, the left diaphragmatic hernia and the dorsal lumbar hernia. × ⅝.

3 Case 2, anterior aspect of the dissected abdominal and pelvic cavities, showing the female internal genital organs, the stenotic rectum, and the umbilical arteries. × ⅝.

4 Anterior aspect of case 2, showing gastroschisis, right talipes calcaneovarus, and the defective left inferior extremity. × ⅓.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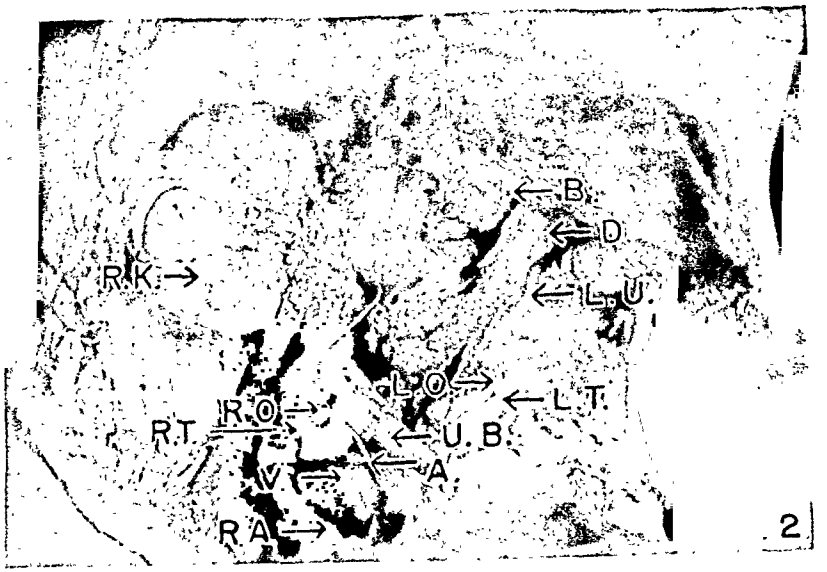
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FURTHER EXPERIMENTS ON THE INTRINSIC CONTRACTILITY OF THE EMBRYONIC RAT HEART [1]

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EIGHT FIGURES

The intrinsic contractility of embryonic mammalian heart muscle in tissue culture preparations has been studied by Goss ('31, '32, '33, '35, '38, '40, '42, '52) and others (review, Patten, '49).

In a previous investigation (Hall, '51), the contraction rates of the two main fragments (sino-atrial and ventriculo-conus) of the embryonic ("nerveless") rat heart were studied. The sino-atrial fragments were found to beat about twice (1.94 ×) as fast as the ventriculo-conus fragments in Ringer solution at 30–32°C.

In the present investigation, similar observations were made at 36.5°C. The sino-atrial fragments were again found to beat about twice (2.28 ×) as fast as the ventriculo-conus fragments.

In addition, sino-atrial fragments were subdivided into sinus and atrial segments, and ventriculo-conus fragments were subdivided into left ventricular, right ventricular and conus segments. The contraction rates of these subdivisions in Ringer solution at 36.5°C. were determined.

It then became evident that under these experimental conditions there is a gradient in the rate of contraction, with the sinus venosus beating the most rapidly, and the atrium, the

[1] Supported in part by a Grant-in-Aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, and in part by a Grant from the Kentucky State Medical Research Commission.

left ventricle, the right ventricle and the conus following in that order.

The experiments serve to elucidate the pace-making mechanism of the tubular stage of the embryonic ("nerveless") rat heart.

MATERIALS AND METHODS[2]

The methods used were in general those of the previous investigation. Pregnant females of the Sprague-Dawley strain of white rats were placed under sodium barbital anesthesia (220–298 mg/kg) supplemented with occasional ether. Depth of anesthesia did not seem to affect the heart rates of the embryos. Nor did long-continued anesthesia appear to affect the embryonic heart rates; typical determinations were made after 10 hours of maternal anesthesia.

The embryos were removed singly at about half-hour intervals from the uterus. The embryos and their hearts and heart fragments were kept in about 100 ml of Ringer solution (composition: .9 gm NaCl, .0235 gm $CaCl_2$, .0456 gm KCl, .02 gm $KHCO_3$, 100 ml H_2O) in a finger bowl, the temperature being maintained at 36.5°C. in a constant temperature bath. The pH of this Ringer before and during the experiment ranged from 8.1 to 8.3; recent observations indicate that the heart rate is not affected by a much wider range of pH variation.

The hearts were excised, subdivided, and studied immediately upon removal of the embryos from the uterus. The 11½ day stage of the embryo was used, since the heart at this stage is still essentially a tube (Hall, '51, fig. 6), though dilatations and constrictions are present which serve to locate the planes of section. Only the boundary between sinus and atrium was difficult to establish, since the sinus at this stage is partly embedded in the septum transversum and liver tissue, and continues without a sharp boundary into the atrium. A V-shaped incision was therefore made to separate sinus and

[2] It is a pleasure to acknowledge the assistance of Mr. Wm. A. Johnson, Mr. B. W. Johnson, and Mr. Raymond L. Rose, Jr. in the course of this investigation.

atrium, to exclude the possibility that sinus tissue might affect atrial beat (fig. 1).

One hundred and sixty-seven hearts and 489 of their subdivisions were studied, their contraction rates being determined by timing 50 beats. All operative procedures on the embryos and their hearts were performed under the binocular microscope at magnifications of 7 ×–30 × with specially sharpened iridectomy scissors and forceps.

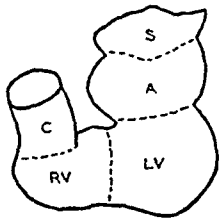

Fig. 1 Diagram of the heart of the 11½ day rat embryo, to show planes of section. S: sinus venosus, A: atrium, LV: left ventricle, RV: right ventricle, C: conus.

The smallness of these subdivisions may be emphasized. The largest ones (left ventricles) were about 0.5 mm in diameter. The wall of the heart tube is highly trabeculated internally at the stage used, and the thickness of the external compact layer does not exceed 45–60 μ.

EXPERIMENTAL

Subdivision of the heart into sino-atrial and ventriculo-conus fragments

Figure 2 is a histogram to show the contraction rates of the sino-atrial (Atrium) and ventriculo-conus (Ventricle) fragments of 50 hearts. It will be observed that there is some overlapping of the curves, as in the previous observations at 30–32° (Hall, '51, fig. 2). The average contraction rate for the 50 sino-atrial fragments was 165.1 per minute, that for the 50 ventriculo-conus fragments 77.5 per minute.

Figure 3 is a histogram to show the distribution of the ratios between the contraction rates of the two fragments

(A/V ratios) of each of the 50 hearts. While there is great variation (from 1.01 to 4.70), there is no case in which the contraction rate of the ventriculo-conus fragment was equal to, or greater than, that of the sino-atrial fragment of the same heart. The average of these 50 A/V ratios is 2.28.

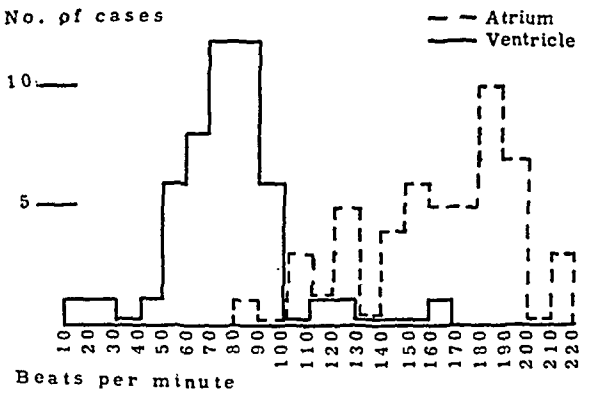

Fig. 2 Histograms to show the contraction rates of the sino-atrial (Atrium) and ventriculo-conus (Ventricle) fragments of 50 hearts.

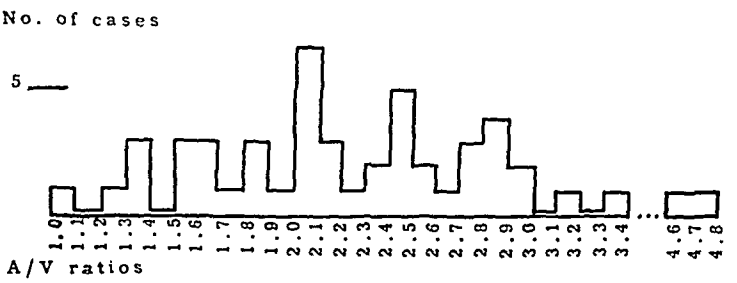

Fig. 3 Histogram to show the distribution of the ratios between the contraction rates (A/V ratios) of the sino-atrial and the ventriculo-conus fragments of 50 hearts.

Subdivision of the sino-atrial fragment into sinus and atrium [3]

Figure 4 is a histogram to show the contraction rates of the sinus and atrial subdivisions of 50 hearts. There is considerable overlapping of the two curves. The average contraction rate for the 50 sinuses was 172.2/minute, but one atrium beat more rapidly (176/minute) than this average.

[3] The following observations were demonstrated by motion picture at the Columbus meeting of the American Association of Anatomists (Anat. Rec., *115*, 447).

The average contraction rate for the 50 atria was 113.9/minute, but two sinuses beat almost as slowly (116 and 118/minute) as this average.

Figure 5 is a histogram to show the distribution of the ratios between the contraction rates of the sinus and the atrial subdivisions (S/A ratios) of each of the 50 hearts. In only one case did the sinus beat more slowly than the atrium of the same heart (137 vs. 140/minute). The average of the 50 S/A ratios is 1.58.

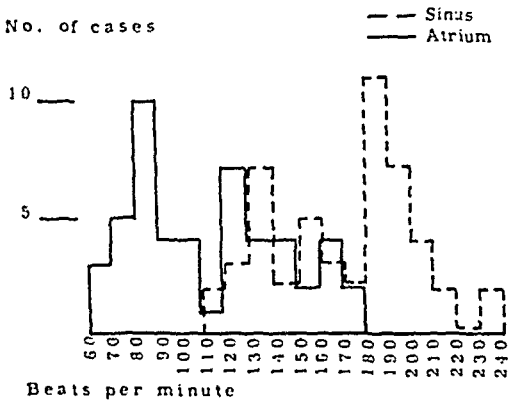

Fig. 4 Histograms to show the contraction rates of the sinus and atrial subdivisions of 50 hearts.

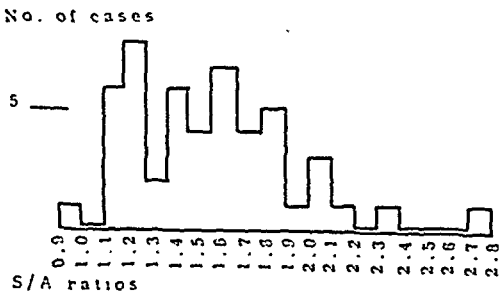

Fig. 5 Histogram to show the distribution of the ratios between the contraction rates (S/A ratios) of the sinus and the atrial subdivisions of 50 hearts.

Subdivision of the ventriculo-conus fragment into left ventricular, right ventricular, and conus subdivisions

The ventriculo-conus fragments were subdivided as shown in figure 1 into left ventricular, right ventricular, and conus

subdivisions. Since these three subdivisions of each heart did not invariably beat within a few minutes after their separation, it was necessary, in order to obtain 50 LV/RV and 50 RV/C ratios, to experiment with 114 hearts, of which only 42 yielded three beating subdivisions of the ventriculo-conus fragment. In all, 239 ventriculo-conus subdivisions were studied.

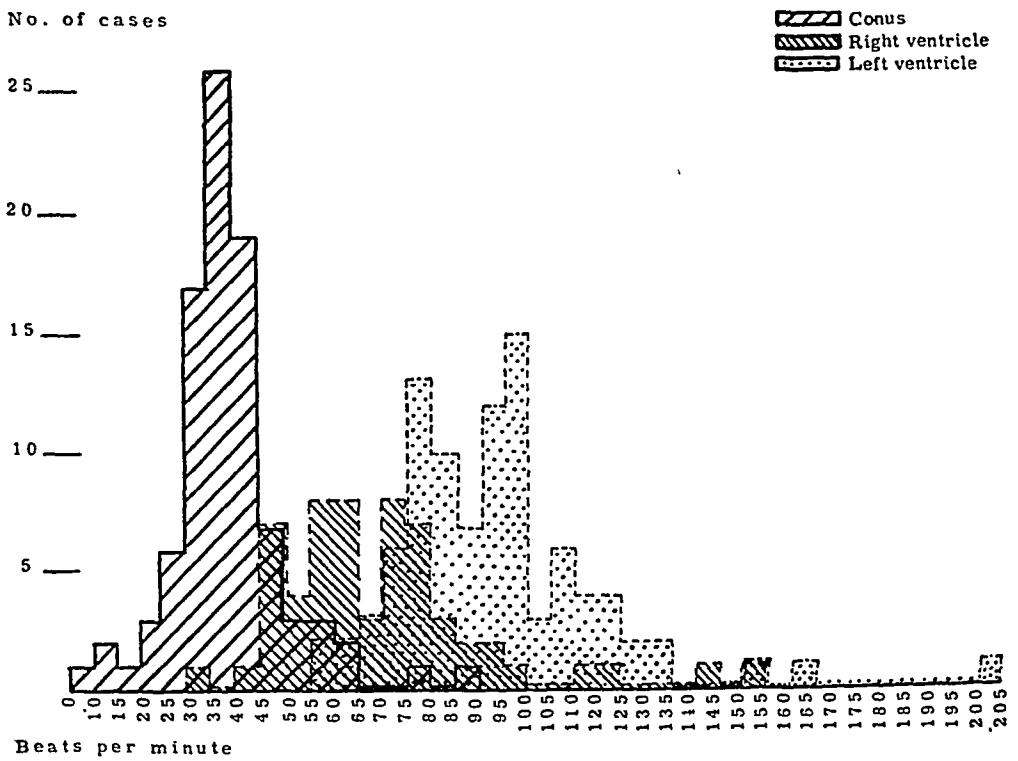

Fig. 6 Histograms to show the contraction rates of 94 left ventricles, 59 right ventricles, and 86 conus subdivisions.

The histograms (fig. 6) to show the contraction rates of the left ventricular, the right ventricular, and the conus subdivisions show some overlapping. The average contraction rate for the 94 left ventricles was 93.9/minute, but 5 of the right ventricles beat faster than this average. The average contraction rate for the 59 right ventricles was 70.7/minute, but two of the conus subdivisions beat faster than this average. The average contraction rate for the 86 conus subdivisions was 39.3/minute.

Figure 7 is a histogram to show the distribution of the ratios between the contraction rates of the left ventricular and the right ventricular subdivisions (LV/RV ratios) of the same heart in 50 cases. In only 4 cases out of 50 did the right ventricle beat faster than the left ventricle of the same

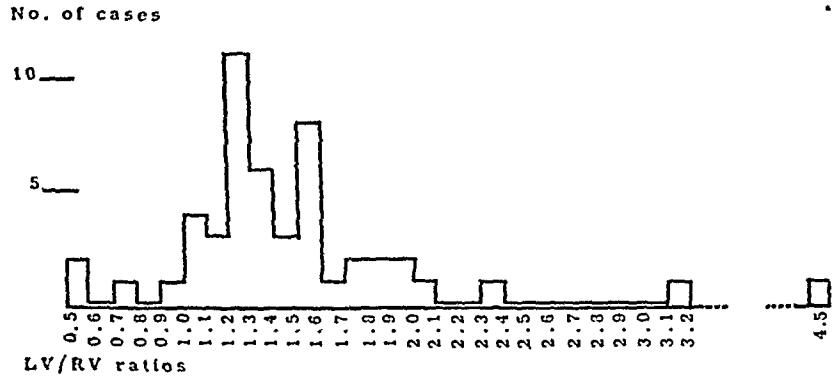

Fig. 7 Histogram to show the distribution of the ratios between the contraction rates (LV/RV ratios) of the left ventricular and right ventricular subdivisions of 50 hearts.

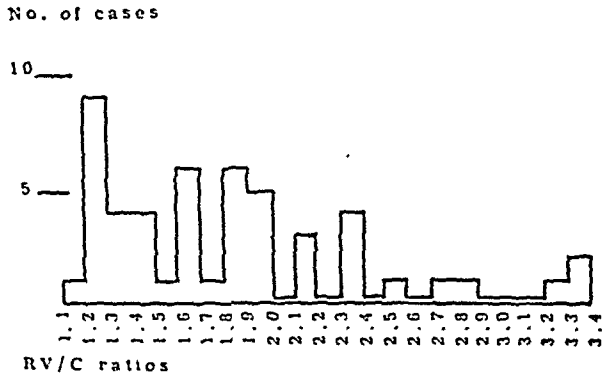

Fig. 8 Histogram to show the distribution of the ratios between the contraction rates (RV/C ratios) of the right ventricular and conus subdivisions of 50 hearts.

heart. In two of these cases, the right ventricular rate was extremely high (139 and 147/minute). In the two other cases, the right ventricular rates were in the upper part of their range, whereas the left ventricular rates were in the lower part of their range. The average of the 50 LV/RV ratios is 1.48.

In no case did the conus beat as fast as, or faster than, the right ventricle of the same heart, as shown by the histogram of the 50 right ventricle/conus (RV/C) ratios (fig. 8). The average of these ratios is 1.84.

DISCUSSION[4]

The present investigation constitutes a confirmation at body temperature of results previously obtained (Hall, '51) at somewhat lower (30–32°C.) temperatures. As in that investigation, the sino-atrial fragments of the embryonic heart were found to beat about twice (2.28 ×) as fast as the ventriculo-conus fragments. The critical ratio (Fisher's t value) for these two distributions is 16.5, indicating that the difference is significant at the 0.1% level, or less.

The present investigation also demonstrated differences in the rate of intrinsic contraction in smaller segments of the heart tube, with the sinus venosus beating most rapidly (172.2/minute) and the succeeding segments beating less and less rapidly (atrium, 113.9/minute; left ventricle, 93.9/minute; right ventricle, 70.7/minute; conus, 39.3/minute).

The critical ratios (t values) for the successive distribution curves are high. That for the sino-atrial values is 9.3, that for the atrio-left ventricular values is 4.5, that for the left ventricular-right ventricular values is 8.8 and that for the right ventricular-conus values is 11.3, indicating in each case that the differences are significant to the 0.1% level, or less. Only in a few exceptional cases did a heart segment beat as fast as, or faster than, the preceding segment of the same heart. The dominance of each segment over the succeeding segment may be inferred.

It was interesting to find, in the course of this investigation, that in most cases the sino-atrial fragment contracted more rapidly than the entire heart from which it was derived. The sinus, in turn, contracted more rapidly than the sino-atrial fragment from which it was derived.

[4] It is a pleasure to acknowledge the assistance of Mrs. Dorothy Hampton Woerner in the computation of the statistical values.

This is not in accord with the observations of Copenhaver ('39) on the embryonic amphibian heart or with those of Barry ('42) on the embryonic chick heart, who found that the sinus rate at various stages did not differ significantly from the rate of the entire heart.

A statistical study was therefore made of 42 hearts, their sino-atrial fragments, and the sinuses derived from these fragments. The mean contraction rate of the 42 entire hearts was 121.6/minute, that of their sino-atrial fragments 132.3, and that of the sinuses derived from these fragments 174.7.

The critical ratio (t value) for the heart-sino-atrial values is 1.74, indicating that the difference is significant to only the 9% level, and we cannot conclude that the difference is significant.

The critical ratio for the heart-sinus values, however, is 8.4, indicating that the difference is significant to the 0.1% level, or less. It may be suggested that the more slowly contracting segments of the heart exert a decelerating influence on the contraction rate of the sinus of the embryonic rat heart.

SUMMARY

This investigation represents a continuation of previous experiments in which 203 hearts of 10–13 day rat embryos were isolated in Ringer at 30–32°C. and sectioned at the atrioventricular level; the contraction rates of the fragments were determined, and the average A/V ratio was found to be 1.94/1.

In the present investigation, these observations were repeated on 50 hearts of the 11 day stage in Ringer at 36.5°C. The average contraction rate for the sino-atrial fragments was 165.1/minute, that for the ventriculo-conus fragments 77.5/minute. The average A/V ratio was 2.28.

The fragments of 11 day hearts were then further subdivided, as follows:

(a) The sino-atrial fragments of 50 hearts were subdivided into sinus and atrial subdivisions. The sinuses con-

tracted at the rate of 172.2/minute, the atria at 113.9/minute. The average S/A ratio was 1.58.

(b) The ventriculo-conus fragments of 114 hearts were subdivided into left ventricular, right ventricular, and conus subdivisions. The average contraction rate for 94 left ventricles was 93.9/minute, that for 59 right ventricles was 70.7/minute, and that for 86 conus subdivisions was 39.3/minute. The average for 50 left ventricle/right ventricle ratios was 1.48. The average for 50 right ventricle/conus ratios was 1.84.

The results indicate that the gradient in the rate of inherent contractility along the embryonic heart tube is a more gradual one than might have been supposed from the previous investigation.

LITERATURE CITED

BARRY, ALEXANDER 1942 The intrinsic pulsation rates of fragments of the embryonic chick heart. J. Exp. Zool., *91:* 119–130.

COPENHAVER, W. M. 1939 Initiation of beat and intrinsic contraction rates in the different parts of the Amblystoma heart. J. Exp. Zool., *80:* 193–224.

GOSS, C. M. 1931 "Slow motion" cinematographs of the contraction of single cardiac muscle cells. Proc. Soc. Exp. Biol. and Med., *29:* 292–293.

———— 1932 The formation of cross-striations in cultures of embryonic heart muscle. Arch. exp. Zellforsch., *12:* 233–248.

———— 1933 Further observations on the differentiation of cardiac muscle in tissue cultures. Arch. exp. Zellforsch., *14:* 175–201.

———— 1935 Double hearts produced experimentally in rat embryos. J. Exp. Zool., *72:* 33–49.

———— 1938 The first contractions of the heart in rat embryos. Anat. Rec., *70:* 505–524.

———— 1940 First contractions of the heart without cytological differentiation. Anat. Rec., *76:* 19–27.

———— 1942 The physiology of the embryonic mammalian heart before circulation. Am. J. Physiol., *137:* 146–152.

———— 1952 Development of the median coordinated ventricle from the lateral hearts in rat embryos with three to six somites. Anat. Rec., *112:* 761–796.

HALL, E. K. 1951 Intrinsic contractility in the embryonic rat heart. Anat. Rec., *111:* 381–399.

PATTEN, BRADLEY M. 1949 Initiation and early changes in the character of the heart beat in vertebrate embryos. Physiol. Rev., *29:* 31–47.

MORPHOLOGIC EFFECTS PRODUCED BY THE IMPLANTATION OF STEROID HORMONE PELLETS NEAR THE HYPOPHYSIS

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TEN FIGURES

INTRODUCTION

While considerable clarification of the physiologic relation between the pituitary and adrenal cortex has been achieved, investigations of pituitary morphology as correlated with adrenocortical activity have been comparatively less rewarding. It has been postulated (Sayers and Sayers, '48) that ACTH secretion is regulated by the blood levels of adrenal cortical hormone. Sayers ('50) has proposed, as a working hypothesis, that the adenohypophysis is itself sensitive to the cortical hormone blood titer and is, therefore, truly autoregulatory.

Attempts have been made to localize the site of "back-action" of the cortical hormones in or near the pituitary, and it has been suggested that the hypothalamus is intimately concerned in regulating pituitary secretion of ACTH (Gershberg, Fry, Brobeck and Long, '50; McDermott, Fry, Brobeck and Long, '50; Hume and Wittenstein, '50; Castor, Baker, Ingle and Li, '51).

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Clinical material has been helpful in studying certain morphologic effects of cortical hormone. The unique hyalinization of basophiles in the pars distalis of Cushing's syndrome, originally described by Crooke, was considered to be "the alteration of fundamental significance in the causation of the syndrome" (Crooke, '35). Although Crooke's observations have been widely confirmed (Kepler, '49), it has been emphasized that the hyaline basophile cannot be regarded as the cause of Cushing's syndrome. The concept that the adrenal cortex is fundamentally and primarily the cause of pituitary basophilism has become increasingly popular (Albright, '43); and, indeed, Golden, Bondy and Sheldon ('50), Laqueur ('51), and Golden and Bondy ('52) have reported the appearance of cells resembling those described by Crooke in the pars distalis following administration of ACTH and adrenal cortical hormone.

It would appear that more positive support of the adrenal causation of Cushing's syndrome and the contention that ACTH is produced by the adenohypophysial basophile would be afforded by a further correlation of morphologic and physiologic effects on the pituitary. Thus, while it is evident that cortical steroids block the release of ACTH (Long, '47; Sayers and Sayers, '47; Gellhorn and Frank, '48; Cheng, '49), there is need for additional evidence that administration of adrenal cortical hormone induces definite changes in pituitary morphology in any subject other than the human. The possibility that basophile cells are the source of ACTH (Koneff, '44; Smelser, '44; Halmi, '50; Marshall, '51) also merits further scrutiny.

As a basis for the present experiments, it was assumed that a satisfactory hormonal field could be established by utilizing the pellet implantation method. Since the quantity of a steroid absorbed from a pellet is regulated by its solubility and surface area and thus can be controlled within reasonable limits, it is possible to introduce into an animal amounts of exogenous hormone that are not far in excess of that animal's physiologic requirements. It should be appar-

ent, however, that a concentration gradient extends away from the undissolved depot of steroid such that the area immediately surrounding the pellet may be considered rich in dissolved hormone as compared with the circulating blood which would contain a relatively low concentration of the substance.

In order to test the hypothesis that adrenal cortical hormone regulates, at least in part, the discharge of ACTH from its secretory source, attempts have been made to create localized fields of hormone activity in and around the pituitary gland. By comparing this experimental treatment with *peripheral* implantation of similar control pellets of active cortical steroid, implantation of pellets of inactive steroids, and prolonged daily injections of large amounts of the same compound, it is possible to draw a distinction between specific hypophysial effects and more general overall metabolic effects. The effects of cortisone have been compared with those of desoxycorticosterone acetate and cholesterol administered in similar fashion. The effectiveness of the method employed has been evaluated by histologic and chemical criteria, including differential cell counts of the pars distalis, analysis of gross and microscopic changes evoked in several organs, assay of adrenal ascorbic acid concentration, and preliminary determinations of pituitary ACTH content. Observations of the changes undergone by the pellets themselves during the course of implantation have also been included.

MATERIALS AND METHODS

A total of 176 adult male and female rats of a closely inbred laboratory strain was used in the experiments. They were kept in a controlled temperature room (24–27°C.) and were maintained on standard laboratory rations consisting of calf meal, meat and bone scraps, cod liver oil, and yeast. All operations were performed on rats weighing 110 to 150 gm. Following operations, animals were given food and water *ad libitum* and were observed at regular intervals for changes in growth and behavior.

The steroids used were cortisone acetate,[3] desoxycorticosterone acetate,[4] estradiol,[4] and cholesterol. Desoxycorticosterone acetate (DCA) and estradiol were procured in the form of 75 mg and 50 mg pellets which were cut into several small pieces, each weighing approximately 1.5 mg, for use in the present experiments. Cortisone acetate and cholesterol were obtained in the form of crystalline powder and were compressed into pellets in a specially machined die.

All implantation operations were carried out using ether anaesthesia. The pituitary region was reached by the parapharyngeal approach; a small hole was drilled through the basisphenoid bone, the dura mater was pierced, and two to 4 pellets totalling approximately 3 mg in weight were inserted through the opening into the sellar region. To insure that the pellets would remain in this location, the hole was plugged with a small piece of the omohyoid muscle which was dissected free during the course of the operation.

Representative animals treated with each of the 4 steroids were sacrificed by exsanguination at intervals of approximately 20, 30, 40, and 60 days postoperatively. Immediately after exsanguination of each animal the left adrenal gland was removed, promptly dissected free of surrounding fat, weighed, and ground thoroughly in a small quantity of sand together with 0.5 ml of 6% trichloracetic acid. Suspensions of whole adrenal tissue were then subjected to analysis for ascorbic acid by a modification of the method of Roe and Kuether ('43). Pituitary, adrenal, thyroid, thymus, spleen, kidney, testis or ovary, and uterus or seminal vesicle weights were determined.

The pituitary, right adrenal, and thyroid glands of all animals were fixed in Helly's fluid and prepared for histologic study. Representative pituitaries were fixed in Champy's fluid following which they were treated by the method de-

[3] Cortone (17-hydroxy-11-dehydrocorticosterone acetate) supplied by the courtesy of Dr. H. J. Robinson and Dr. A. Gibson of Merck and Co., Inc.

[4] Cortate (desoxycorticosterone acetate) and Progynon (estradiol) kindly supplied by Dr. N. L. Heminway of Schering Corp.

scribed by Severinghaus ('32) for study of mitochondria and Golgi apparatus. After they had been embedded in paraffin and serially sectioned in the horizontal (frontal) plane at 4 μ, the pituitaries which had been fixed in Helly's fluid were stained by a modification of Masson's method.

Differential counts of the three basic cell types of the pars distalis stained by the preceding method were determined by a routine similar to that devised by Kirkman ('37). The counting chamber was a Whipple micrometer disc containing one large square ruled into 100 smaller squares; the actual length of one side of the large square is 6.9 mm.

In each pituitary the first section to be counted was chosen near the beginning of the series of adenohypophysial sections, and, starting at that point, every 50th section throughout the series was used for the count. Each of the sections selected for counting was observed under oil immersion. In all pituitaries which were subjected to this procedure the final cell counts were based upon at least three sections of the gland. In every case, percentages of the cell types were calculated from a total of at least 2500 counted cells, and, in most instances, more than 5000 cells were counted. While no precise overall measurements of cell mass were made, sizes of various cells were noted in some detail.

Whenever practicable, the pellets were removed from animals at autopsy, dried in a desiccator, and weighed. A rough estimate of the amount of steroid absorbed daily during the period of administration was derived by subtracting the final from the initial pellet weight and dividing this figure by the total number of days of implantation.

Differential cell counts were also performed on pituitaries taken from animals which had received, by subcutaneous injection, 2 to 9 mg of cortisone acetate in 0.5 ml of 0.85% NaCl solution daily for a period of two weeks.

Animals which received pellet implants of cortisone or of DCA in the vicinity of the pituitary were grouped as the experimental series. Control groups consisted of rats that had received pellet implants of cholesterol in the pituitary

region and animals that were implanted in the groin regions with pellets of similar size and weight for each of the steroids studied. Pellets of estradiol were also implanted in the sellar area to provide information concerning the absorption properties of another well-known steroid. Physiologic effects of this hormone administered in this manner will not be considered in the present report.

Statistical analyses were performed according to procedures outlined by Snedecor ('46).

RESULTS

1. Effects on the intact animal. Daily measurements of body weight, food and water consumption, and urine volume revealed no marked differences between control and experimental animals.

2. Effect on organ weights. Since no appreciable differences were determined in the weights of organs of animals sacrificed at different lengths of time after pellet implantation, these data have been pooled for the purpose of statistical evaluation (table 1); that is, all animals subjected to each kind of experimental treatment have been considered as a discrete group. The data for the organ weights in the males are not given since no significant alterations were noted with the various treatments used.

In female rats the pituitaries which were exposed to pellets of cortisone were reduced in weight to a statistically significant degree as compared with the pituitaries of animals treated with cortisone pellets in the groin, cholesterol or DCA pellets near the pituitary, and normal untreated animals. The principal source of the highly significant variance ratio, $F = 4.05^{**}$ (table 1) is shown, by the comparison of individual groups (Student's t test), to be the females implanted with cortisone pellets near the pituitary ($p < .001$). A reduction in weight of the male pituitary exposed to cortisone is also suggested by the data, but to a lesser and statistically insignificant extent.

TABLE 1

Statistical analysis of organ weights of female rats implanted with steroid pellets

TREATMENT	NO. RATS	BODY WEIGHT	PITUITARY	ADRENALS	THYROIDS	OVARIES	UTERUS	THYMUS	SPLEEN	KIDNEYS
		gm	*mg/100 gm*	*mg/100 gm*	*mg/100 gm*	*mg/100 gm*	*mg/100 gm*	*mg/100 gm*	*mg/gm*	*mg/gm*
None	17	168	6.09 ± 0.22	23.2 ± 0.54	9.18 ± 0.33	29.4 ± 1.55	208 ± 15.1	106.8 ± 8.7	3.75 ± 0.33	6.42 ± 0.16
Cortisone in pituitary	7	164	4.11** ± 0.36	22.8 ± 1.27	10.10 ± 0.63	29.3 ± 2.89	189 ± 12.5	136.0 ± 13.0	3.38 ± 0.21	6.41 ± 0.20
Cortisone in groin	8	166	5.71 ± 0.24	24.9 ± 1.46	7.90 ± 0.50	27.7 ± 1.35	161 ± 14.1	119.0 ± 12.1	3.54 ± 0.24	6.56 ± 0.23
DCA in pituitary	7	158	5.21 ± 0.85	26.3 ± 1.40	9.65 ± 0.81	30.0 ± 1.52	204 ± 14.4	129.0 ± 9.1	4.08 ± 0.22	6.98 ± 0.11
Cholesterol in pituitary	5	165	5.85 ± 0.74	26.1 ± 1.30	12.10* ± 0.24	34.4 ± 5.53	165 ± 16.2	149.0 ± 13.1	3.39 ± 0.33	7.05 ± 0.49
F			4.05†	2.21	7.09†	0.73	1.61	2.17	0.55	1.51

Figures represent means ± standard error. Each value of F represents the variance ratio, $\frac{\text{Mean square of group means}}{\text{Mean square of individuals}}$, where the numerator includes all treatment groups listed in column 1.

* $p < .05$, indicating significant difference from control value in first line of table (Student's t test).

** $p < .01$.

† $p < .01$, indicating significant heterogeneity within group means (analysis of variance).

In neither male nor female was there any significant alteration in weight of the adrenal glands, gonads, sexual accessory organs, spleen, or kidneys as a result of cortisone treatment, either of the experimental or control type. Simultaneous comparison of the female groups disclosed a significant difference in thyroid weights, $F = 7.09^{**}$ (table 1). By inspection of the data, this may be attributed to the cholesterol treatment.

3. Adrenal ascorbic acid. The concentration of ascorbic acid in the whole adrenal gland of both the male and the female was not significantly affected by any of the treatments applied, as indicated by the variance ratios, $F = 0.56$ and $F = 2.23$ (from statistical analysis of the 5 groups of males and females in the same manner as presented in table 1). The untreated female gland contained less ascorbic acid than did the normal male, a mean difference of 90.2 mg per 100 gm adrenal weight ($p < .001$).

4. Histology of the endocrine organs. The microscopic structure of the adrenal cortex, thyroid gland, and gonads was unaffected by treatment with either cortisone or DCA pellets. The cells of each of the adrenal cortical zones displayed a normal degree of vacuolization, and the zonal widths were within the normal range. The heights of the thyroid cells fell within the normal range, and the follicles were filled with varying amounts of colloid. Gametogenic activity did not appear to be affected in either the testis or the ovary.

5. Pituitary histology.

(a) Normal. The present study is based on a differentiation of three classes of cells: acidophile, basophile, and chromophobe.

The male and female adenohypophysis may be said to be alike in certain qualitative aspects, such as shape and distribution of cell types. Quantitatively, however, they are significantly different. The male gland (table 2) contains a higher proportion of basophiles and acidophiles than the female (table 3). There appears to be little variation among

the different untreated animals of each sex with regard to the proportions of cell types.

There was no evidence of mitotic activity in any of the cells of any of the normal or experimental hypophyses studied.

TABLE 2

Percentages of cell types in male pituitary glands

TREATMENT	NO. OF GLANDS COUNTED	TOTAL NO. OF CELLS COUNTED	% ACIDOPHILES	% BASOPHILES	% CHROMOPHOBES
None	6	38,489	36.5 ± 0.64	10.6 ± 0.62	52.9± 0.55
Cortisone in pituitary	8	43,092	32.4 ± 1.42*	15.9 ± 2.10*	51.7 ± 1.45
Cortisone in groin	5	33,298	36.1 ± 0.93	9.4 ± 0.20	54.6 ± 1.18
DCA in pituitary	4	20,123	35.9 ± 0.94	10.2 ± 0.99	54.0 ± 0.91

Cell type per cent figures are the means ± their standard error.

Figures followed by (*) are significantly different ($p < .05$) from the appropriate control values found in the first line of the table (Student's t test).

TABLE 3

Percentages of cell types in female pituitary glands

TREATMENT	NO. OF GLANDS COUNTED	TOTAL NO. OF CELLS COUNTED	% ACIDOPHILES	% BASOPHILES	% CHROMOPHOBES
None	5	30,732	29.8 ± 1.22	7.5 ± 0.61	62.7 ± 1.60
Cortisone in pituitary	5	16,768	29.2 ± 2.08	13.7 ± 1.69**	57.1 ± 1.37*
Cortisone in groin	4	24,528	30.5 ± 2.48	8.0 ± 0.38	61.5 ± 2.26
DCA in pituitary	4	18,330	31.6 ± 1.78	7.8 ± 0.42	60.6 ± 1.45
Cortisone injected sub-cut.	5	32,117	29.9 ± 0.95	13.4 ± 1.56**	56.8 ± 1.68*

Cell type per cent figures are the means ± their standard errors.

Figures followed by (*) are significantly different ($p < .05$) from the appropriate control values found in the first line of the table (Student's t test).

** Denotes $p < .01$.

(b) Experimental. The animals bearing sellar implants of cortisone present definite morphologic evidence of the influence of this hormone on adenohypophysial cells. The most profound changes are to be found in the basophiles. Most typically, those cells become hypertrophied (fig. 5). In such enlarged cells, the granulation stains with normal intensity, but in a few cases these basophilic cytoplasmic granules appear concentrated, or clumped, throughout the cytoplasmic matrix (figs. 3 and 4). Most characteristic of the cortisone-altered basophile, however, is the development of the large intracellular vacuole (figs. 3, 5, and 8). Usually only one vacuole is found in each cell. It is characteristically spherical and occupies a large portion of the entire cell. The edge of the vacuole clearly demarcates its interior from the cytoplasmic substance and, in most cases, the vacuole is filled with a light to intensely basophilic hyaline substance. Vacuoles which are found in basophiles of the normal gland remain unaffected by the stain. Such colorless vacuoles are sometimes seen within the hyaline vacuoles of the cortisone pellet-altered basophile (fig. 5). The basophile cell which results from cortisone treatment resembles the "signet-ring" cell associated with castration.

The Golgi apparatus of the cell affected in this manner gives little or no indication of constant alteration (figs. 7 and 8). It appears, however, clearly defined and occupies a zone of the cytoplasm that is well differentiated and separated from the nucleus. The negative Golgi image is frequently observed in Masson stained sections, more in the experimental than in the normal. The picture described is found with equal frequency in males and females.

In many cases the sections of anterior lobe present a decidedly basophilic overall appearance. In such instances there is an abundance of small, lightly basophilic cells which are here presumed to represent chromophobes that have been altered to assume at least some staining properties of basophiles. Such cells have been classified as transitional basophiles. It is felt that the chromophobic origin of these cells

is indicated by their size, scanty granulation, and syncytium-like arrangement. At times, cells such as these seem detached from their neighbors and appear more definitely as basophiles.

The acidophile shows little effect of the cortisone pellets placed near the pituitary. In many of these glands, though, acidophilic granulation is qualitatively reduced. A somewhat extreme and infrequent example of this observation is to be found in cells which may retain but a narrow rim of acidophilic granules around the periphery of the cytoplasm. The distribution of acidophiles remains normal as far as could be ascertained.

There appears to be no tendency of the observed changes to differ in magnitude, either qualitatively or quantitatively, with time; that is, pituitaries taken after 20 days of direct exposure to cortisone present essentially the same picture as those exposed for 60 days.

While the pituitaries of animals that had been *injected* with large doses of cortisone may be said definitely to be basophilic, the pattern of this basophilia is of the type, described above, of the altered chromophobe, cells which are small and which seem to merge almost imperceptibly with their basophilic or chromophobic neighbors. There is no enlarged basophile comparable to the cell found in the pellet-treated gland and vacuolization occurs with no unusual frequency; in several sections no vacuoles at all were observed. The staining affinity of these basophiles is less than that of any of the other glands. The degree of granulation of acidophiles is definitely decreased so that such cells resemble those found in some of the pellet-treated animals.

In addition to the qualitative changes in appearance of basophiles, significant quantitative alterations (tables 2 and 3) may be summarized as follows: the proportion of basophiles in both males and females that had received juxtapituitary implants of cortisone rose markedly. Acidophiles decreased in male rats with juxtapituitary implants of cortisone; no such change was apparent in the females treated in the same way. The only other group that showed any

TABLE 4

Absorption from steroid pellets implanted for periods ranging from 20 to 60 days

STEROID	SITE OF IMPLANTATION	NO. OF PELLETS *	INITIAL PELLET WEIGHT *	FINAL PELLET WEIGHT *	MEAN ABSORPTION RATE	MEDIAN ABSORPTION RATE
			mg	*mg*	*µg/day*	*µg/day*
Cortisone	Pituitary	8	3.28 ± 0.05	0.64 ± 0.21	57 ± 5	59
Cortisone	Groin	6	2.52 ± 0.14	0.68 ± 0.21	63 ± 4	66
DCA	Pituitary	6	2.58 ± 0.33	1.15 ± 0.35	49 ± 7	46
Estradiol	Pituitary	8	2.21 ± 0.19	1.26 ± 0.22	32 ± 5	26
Cholesterol	Pituitary	4	4.93 ± 0.77	4.25 ± 0.82	26 ± 13	18

* Each implant is regarded here as a single pellet, even though several fragments might be included (either as a result of implantation in that form or from fracture in recovery of the implanted material). Both mean and median absorption rates calculated from individual absorptions, initial weight minus final weight/days of implantation.

± Figures are standard errors.

significant alteration in the proportion of either of the chromophilic components was the cortisone-injected female in which a significant increase was noted in the percentages of basophiles.

6. Pellets. Pellet residues were recovered when animals of each of the several groups were sacrificed. This was not always possible since many of the implanted pellets were known to have fragmented during the course of implantation. In addition, small bits of pellet were on occasion found so deeply imbedded in the pituitary that their removal by dissection would have destroyed excessive amounts of anterior pituitary tissue intended for histologic examination.

The estimations based on initial and final pellet weight differences indicate (table 4) that cortisone was absorbed to the extent of approximately 60 μg per day. The difference between the amount absorbed from cortisone pellets in the pituitary as compared with those in the groin was not appreciable. DCA, however, was not as rapidly absorbed, and estradiol and cholesterol, respectively, were absorbed still more slowly.

A characteristic structure was presented by pellet ghosts which were prepared by subjecting residual pellets, recovered after 25 days of implantation, to fixation and treatment which effectively removed all remaining steroid. The most prominent feature here was the extensive pellet capsule (figs. 9 and 10). This consisted almost entirely of a dense network of collagenous connective tissue fibers which completely surrounded the former steroid depot. The sheath formed about cholesterol pellets was considerably thicker and heavier than that which developed around cortisone pellets of similar initial size and weight. Although the capsular thickness varied from point to point, it averaged approximately 31 μ for cholesterol; the cortisone capsule was, on the average, 7 μ in thickness. Aside from a very few strands of noncellular material extending across the residual lumen, these ghosts gave no evidence of any internal structure.

DISCUSSION

It is to be emphasized that in no instance did cortisone or DCA pellet implantation evoke any apparent effects on the organs studied, with the exception of the hypophysis. On the other hand, significant alterations are induced in body organs following chronic parenteral administration of adrenal steroids (Carnes, Ragan, Ferrebee and O'Neill, '41; Winter, Silber and Stoerck, '50; Antopol, '50).

It is generally acknowledged that the effects of large doses of cortical hormone are mediated by either or both of two mechanisms. The first is a direct one, that is, the administered steroid evokes certain definite alterations of the normal metabolic pattern, examples of which would be inhibition of growth, production of negative nitrogen balance, and increase in liver glycogen deposition. The second mechanism is an indirect one and involves the intermediation of the pituitary. Thus, the atrophy of the adrenals of animals treated with cortisone or with adrenal cortical extract may be attributed, at least in large part, to blockade of ACTH discharge from the adenohypophysis. The absence of any cortisone or DCA pellet effects on general metabolism as demonstrated in the present work indicates that the quantities of steroids entering the circulation by absorption from the pellets were small (table 4) and did not, therefore, exceed the limits of the "physiologic range" of cortical steroid concentration for the organism as a whole.

Since the secretion of adrenal steroids depends, in large part, on environmental conditions at any given time (Sayers, '50; Selye, '51), it is evident that it would be exceedingly difficult to assess the physiologic level of adrenal cortical hormone in any "normal" animal, because of the inherent variability of this factor.

Sayers ('50), however, has estimated in the rat a range of 30 to 400 μg of "cortisone equivalent" necessary per eucortical animal per day. On the other hand, in stress the daily requirement may reach 4000 μg of "cortisone equivalent" necessary per animal per day. It is, nonetheless, impossible to

calculate from these figures the daily production of adrenal cortical steroids, since the rapidity of metabolism of the hormone or its rate of utilization cannot be measured. Although the figures (table 4) indicating absorption rates are admittedly crude and not without error as a result of invasion of pellets by insoluble protein material during the course of implantation, they are sufficient, nevertheless, to indicate the approximate amount of exogenous steroid available by absorption per animal per day.

It may be inferred from the lack of effect on organ weight and histology that the pellets of cortisone placed next to the pituitary were not effective in elevating the blood titer of cortical steroid far beyond that found in the eucortical rat; but, by virtue of the concentration gradient established around the pellet, only the pituitary was exposed to abnormally large quantities of the hormone.

That the reduction in pituitary mass resulting from cortisone pellet implantation in the sellar region is a true one and not produced by the operative procedures is demonstrated by the fact that neither cholesterol nor DCA produced comparable weight decreases. Qualitatively similar pituitary weight reductions have been produced by large doses of parenterally administered DCA (Carnes et al., '41) and by large doses of cortisone (Antopol, '50).

With such an appreciable loss in pituitary weight, it might be expected that less trophic hormones would be produced by such a gland, approximately only two-thirds the normal size. Smith ('32) has shown that animals will survive after partial hypophysectomy with complete maintenance of gonad, thyroid, and adrenal cortical function, even after as much as 70% of the hypophysis has been removed. The loss of pituitary tissue does not attain this magnitude even in the animals contributing to the lower tail of the distribution of pituitary weights. It is obvious, then, that even the subnormal amounts of anterior pituitary in the females which had borne sellar implants of cortisone pellets were sufficient to main-

tain a normal level of function in those organs dependent upon the trophic hormones.

The most striking evidence of alteration of basophilic activity was the appearance of the enlarged vacuolated cell peculiar to the hypophyses that had been adjacent to implanted pellets of cortisone. This type of basophile was not found in either normal animals, DCA-treated animals, cholesterol pellet-treated animals, or animals that had been implanted with cortisone pellets in the groin. It is important to note that none of the 6 animals which received varying amounts of cortisone, from 2 to 10 mg daily, by subcutaneous injection, gave any indication of this qualitative type of basophile alteration. The pituitary differential counts of these rats indicate, however, a higher incidence of basophiles than normal, but these were mainly of the transitional type.

The only changes apparent in the acidophiles were quite variable and of a qualitative nature. Depletion of acidophilic granulation was seen in the pituitaries of both males and females that had received sellar implants of either cortisone or DCA. It is difficult to relate these granular differences with any disturbance of normal secretory activity because of the fact that they were found in only a few glands and no constant pattern of their incidence was discernible.

The altered basophile resulting from the implantation of cortisone pellets near the pituitary resembles most closely the cytologic picture produced by castration. The signet ring cell produced by castration in the rat may be said to represent an altered *delta cell* (Halmi, '50) or *gonadotroph* (Purves and Griesbach, '51). The resemblance between the basophiles subjected to the cortisone field and the signet ring cell of castration is unmistakable. The two cells are alike in the extent of the hyaline vacuole, the crowding of the cytoplasm into a narrow zone, and the peripheral clumping of basophilic granulation. In the present experiments the picture appeared after staining by Masson's method, while Purves and Griesbach's ('51) observations were based on the periodic acid Schiff method of visualization. Because of this difference in

technical treatment of the pituitary sections, it is not possible to state that the two changes are in reality one and the same. It may be that the similarity is purely fortuitous, since the implantation of cortisone pellets near the pituitary would be expected to have the effect of disturbing the ACTH-adrenal cortical relationship, and the periodic acid Schiff reaction identifies glycoprotein. Although gonadotrophin is thought to be glycoprotein (Catchpole, '49), ACTH cannot be regarded as this type of compound (Li, '51). Laqueur's ('51) observation, however, that both the granular and hyaline material of the Crooke's cells in the human pituitary are periodic acid Schiff positive, suggests that ACTH may be related in some way to glycoprotein within the site of its synthesis. The Golgi apparatus is one of the cytologic features that indicates a difference between the castration cell and the cells observed after implantation of cortisone pellets near the pituitary. While the castration cell contains an hypertrophied, vesiculated Golgi body which has been interpreted as an indication of a greatly heightened elaboration of secretion (Severinghaus, '37), the Golgi of the basophile resulting from juxtapituitary cortisone implantation does not appear to be very different from that of the normal. It is suggested that the alterations observed in the basophiles subjected to cortisone by diffusion from pellets involve a disturbance in some step or steps in the synthesis of trophic hormone(s) which might eventually lead to a decrease in the amount released from the adenohypophysis. The mechanism underlying this process cannot be identified, but Severinghaus' idea of the production of vacuolization by the force of an abnormal secretory "back-pressure" is an attractive one. The absence of detectable changes in the adrenal cortex of the animals that had received sellar implants of cortisone pellets may be due possibly to an inadequacy of the methods used for assessing adrenal cortical activity or to an insufficient degree of inhibition of ACTH release.

A preliminary series of ACTH assays, performed in an attempt to test the hypothesis, did not indicate any detectable

differences in ACTH content between cortisone-implanted and untreated pituitaries. Halmi and Bogdanove ('51a, '51b) similarly have been unable to demonstrate any significant change in ACTH content of glands altered either by castration or thyroidectomy, nor could Golden and Bondy ('52) reconcile cortisone-induced pituitary changes with demand for endogenous ACTH. The suggestion by both groups of authors that, using available methods, it is difficult to relate adenohypophysial morphology to functional activity is strengthened by the present results.

It is suggested, on the basis of the available evidence, that the picture of the cortisone altered basophile in the present experiments may be the murine counterpart of the alteration of the human basophile originally described by Crooke ('35) and recently studied in the rat (Golden and Bondy, '52).

Observations in the present experiments are in agreement with the findings of Folley ('42) and of Deanesley and Parkes ('43) that implanted pellets become encapsulated and infiltrated with a meshwork of protein. While the pellet method of hormone administration has been limited by the decrease in absorption produced by connective tissue encapsulation, most of the available information on capsule formation has been obtained using sex steroids. The growth-inhibiting properties of 11-oxygenated adrenal steroids have been demonstrated in skin and wound healing, and the concept that growth depression may be caused by local inhibitory action of cortisone is supported by the findings of Meier, Schuler and Dessaulles ('50). It may be expected, therefore, that pellet encapsulation would not develop as a serious deterrent to cortisone absorption. While such encapsulation was not completely prevented by cortisone, it was materially reduced as judged by comparison of the sectioned cortisone and cholesterol pellet residues.

SUMMARY

The relation of the adrenal cortical steroids, cortisone and 11-desoxycorticosterone to the adenohypophysis has been in-

vestigated by employing the hormonal field approach. The placing of cortisone pellets next to the pituitary of the male and female rat produced quantitative changes in the proportions of cell types of the pars distalis. The relative numbers of basophiles were increased in both sexes, and this has been attributed to the increased frequency of transitional basophiles. Acidophiles were altered only slightly and variably.

The basophiles were observed to have undergone striking and extensive alterations characterized by total hypertrophy, development of large hyaline vacuoles, and peripheral aggregation of the cytoplasmic granulation.

The pituitary itself was significantly reduced in size in the female only by the experimental cortisone treatment. There were no detectable effects upon the organ weights and histology of the several target organs studied. The conclusion is reached that a cortical hormone field was effectively established within the hypophyses that were exposed to cortisone pellet implants.

The degree of development of a connective tissue capsule and ghost by the pellets has been studied and related to the steroid composition of the pellet.

The present results are consistent with the hypothesis that the basophile is the cellular source of synthesis of adrenocorticotrophin. The data support the contention that the adrenal cortex is primary, relative to the adenohypophysis, in the etiology of Cushing's syndrome.

LITERATURE CITED

ALBRIGHT, F. 1943 Cushing's syndrome; its pathological physiology, its relationship to the adreno-genital syndrome, and its connection with the problem of the reaction of the body to injurious agents ("alarm reaction" of Selye). Harvey Lect., *38:* 123–186.

ANTOPOL, W. 1950 Anatomic changes produced in mice treated with excessive doses of cortisone. Proc. Soc. Exper. Biol. and Med., *73:* 262–265.

CHENEY, W. H., C. RAGAN, J. W. FERREBEE AND J. O'NEILL 1941 Effects of desoxycorticosterone acetate in the albino rat. Endocrinology, *29:* 144–149.

CASTOR, C. W., AND B. L. BAKER 1950 The local action of adrenocortical steroids on epidermis and connective tissue of the skin. Endocrinology, *47:* 234–241.

CATCHPOLE, H. R. 1949 Distribution of glycoprotein hormones in the anterior pituitary gland of the rat. J. Endocrinol., *6:* 218–226.

CHENG, C. P. 1949 Regulation of pituitary adrenocorticotrophic activity by adrenal cortical hormones. J. Clin. Endocrinol., *9:* 656.

CROOKE, A. C. 1935 A change in the basophil cells of the pituitary gland common to conditions which exhibit the syndrome attributed to basophil adenoma. J. Path. and Bact., *41:* 339–349.

DEANESLY, R., AND A. S. PARKES 1943 Deposition of protein material in implanted pellets of steroid hormones. Lancet, *245* (2): 500–502.

FOLLEY, S. J. 1942 Retarding effect of ghost formation on absorption from subcutaneously implanted tablets of hexoestrol. Nature, *150:* 735–736.

GELLHORN, E., AND S. FRANK 1948 Sensitivity of the lymphopenic reaction to adrenalin. Proc. Soc. Exper. Biol. and Med., *69:* 426–429.

GERSHBERG, H., E. G. FRY, J. R. BROBECK AND C. N. H. LONG 1950 The role of epinephrine in the secretion of the adrenal cortex. Yale J. Biol. and Med., *23:* 32–51.

GOLDEN, A., AND P. K. BONDY 1952 Cytologic changes in rat adenohypophysis following administration of adrenocorticotrophin or cortisone. Proc. Soc. Exper. Biol. and Med., *79:* 252–254.

GOLDEN, A., P. K. BONDY AND W. H. SHELDON 1950 Pituitary basophile hyperplasia and Crooke's hyaline changes in man after ACTH therapy. Proc. Soc. Exper. Biol. and Med., *74:* 455–458.

HALMI, N. S. 1950 Two types of basophils in the anterior pituitary of the rat and their respective cytophysiological significance. Endocrinology, *47:* 289–299.

HALMI, N. S., AND E. M. BOGDANOVE 1951a Effect of thyroidectomy on ACTH content of rat adenohypophysis. Proc. Soc. Exper. Biol. and Med., *77:* 518–520.

———— 1951b Effect of estrogen treatment and castration on ACTH content of rat adenohypophysis. Proc. Soc. Exper. Biol. and Med., *78:* 95–97.

HUME, D. M., AND G. J. WITTENSTEIN 1950 The relationship of the hypothalamus to pituitary-adrenocortical function. In: Proceedings of the First Clinical ACTH Conference, John R. Mote, ed. The Blakiston Co., Philadelphia, pp. 134–147.

KEPLER, E. J. 1949 Cushing's disease: a primary disorder of the adrenal cortices? Ann. New York Acad. Sci., *50:* 657–678.

KIRKMAN, H. 1937 A cytological study of the anterior hypophysis of the guinea pig and a statistical analysis of its cell types. Am. J. Anat., *61:* 233–288.

KONEFF, A. A. 1944 Effect of adrenocorticotropic hormone on the anterior pituitary of the normal young male rat. Endocrinology, *34:* 77–82.

LAQUEUR, G. L. 1951 Pituitary changes in diseases of the adrenal cortex. Am. J. Path., *27:* 707–708.

LI, C. H. 1951 The present status of the chemistry of ACTH. In: Transactions of the Second Conference, Josiah Macy, Jr. Foundation, E. Ralli, ed. Prog. Assn., Caldwell, N. J., pp. 11–47.

LONG, C. N. H. 1947 The relation of cholesterol and ascorbic acid to the secretion of the adrenal cortex. Recent Progress in Hormone Research, *1:* 99–122.

MARSHALL, J. M. 1951 Localization of adrenocorticotrophic hormone by histochemical and microchemical methods. J. Exp. Med., *94:* 21–30.

MEIER, R., W. SCHULER AND P. DESAULLES 1950 Zur Frage des Mechanismus der Hemmung des Bindegewebstums durch Cortisone. Experientia, *6:* 469–471.

MCDERMOTT, W. V., E. G. FRY, J. R. BROBECK AND C. N. H. LONG 1950 Mechanism of control of adrenocorticotrophic hormone. Yale J. Biol. and Med., *23:* 52–66.

PURVES, H. D., AND W. E. GRIESBACH 1951 The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoprotein. Endocrinology, *49:* 244–264.

ROE, J. H., AND C. A. KUETHER 1943 The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem., *147:* 399–407.

SAYERS, G. 1950 The adrenal cortex and homeostasis. Physiol. Rev., *30:* 241–320.

SAYERS, G., AND M. A. SAYERS 1947 Regulation of pituitary adrenocorticotropic activity during the response of the rat to acute stress. Endocrinology, *40:* 265–273.

———— 1948 The pituitary-adrenal system. Recent Progress in Hormone Research, *2:* 81–115.

SELYE, H. 1951 General adaptation syndrome and the diseases of adaptation. Amer. J. Med., *10:* 549–555.

SEVERINGHAUS, A. E. 1932 A cytological technique for the study of the anterior lobe of the hypophysis. Anat. Rec. *53:* 1–5.

———— 1937 Cellular changes in the rat pituitary with special reference to its secretory activities. Physiol. Rev., *17:* 556–588.

SMELSER, G. K. 1944 Differential concentration of hormones in the central and peripheral zones of the bovine anterior pituitary gland. Endocrinology, *34:* 39–45.

SMITH, P. E. 1932 The secretory capacity of the anterior hypophysis as evidenced by the effect of partial hypophysectomy in rats. Anat. Rec., *52:* 191–207.

SNEDECOR, G. W. 1946 Statistical methods. 4th ed., Iowa State College Press, Ames, Iowa.

WINTER, C. A., R. H. SILBER, AND H. C. STOERCK 1950 Production of reversible hyperadrenocortinism in rats by prolonged administration of cortisone. Endocrinology, *47:* 60–72.

PLATE 1

EXPLANATION OF FIGURES

Figures 1–5 are from pituitaries fixed in Helly's fluid, sectioned at 4 μ, and stained by a modification of Masson's method.

1 Section through typical field of pars distalis of a normal adult male rat. × 760.

2 Another area from normal male rat pars distalis. The group of large, darker cells is representative of normal basophiles; negative Golgi images are visible in the two larger cells. × 1290.

3 Section through pars distalis of male pituitary that had been adjacent to cortisone pellet for 30 days. Note enlarged basophiles with aggregated granules and central hyalinized regions. × 760.

4 Higher magnification of another field of same gland as in figure 5. The peripheral clumping of basophilic granules is most apparent. × 1290.

5 Section of pars distalis exposed to cortisone for 60 days. Hyaline vacuoles may be seen within the cytoplasm of the hypertrophied basophiles. Note the small, unstained, vacuolar spaces within the hyaline substance. × 1290.

STEROID HORMONE EFFECTS ON PITUITARY

ROBERT F. KALLMAN AND ALBERT S. GORDON

PLATE 2

EXPLANATION OF FIGURES

6 Dorsal view of head of rat that had been implanted with cortisone (C) near the hypophysis (H). The brain has been removed to expose the hypophysial region. × 1.33.

7 Section of pars distalis of normal adult male rat, showing Golgi apparatus of acidophiles (capping nucleus) and basophiles (separate from nucleus). Champy fixation, osmium tetroxide for 5 days, sectioned at 3 μ, unstained. × 1290.

8 Section from pars distalis of pituitary exposed to cortisone pellet for 50 days. Note large basophile containing spherical clear vacuole (V) flanked by smaller nucleus (N) and dark Golgi body (G). Mitochondria are abundant. Champy fixation, osmium tetroxide for 5 days, sectioned at 3 μ, unstained. × 1290.

9 Transverse section of cholesterol pellet implanted in groin for 25 days. The steroid has been removed from this and pellet shown in figure 10 by dissolving in absolute alcohol. Compare thick collagenous capsule around steroid site with that formed around cortisone in figure 10. Formalin fixation, Masson stain. × 47.

10 Cortisone pellet implanted in groin for 25 days. This and the pellet shown in figure 9 were initially of the same diameter. Formalin fixation, Masson stain. × 47.

STEROID HORMONE EFFECTS ON PITUITARY

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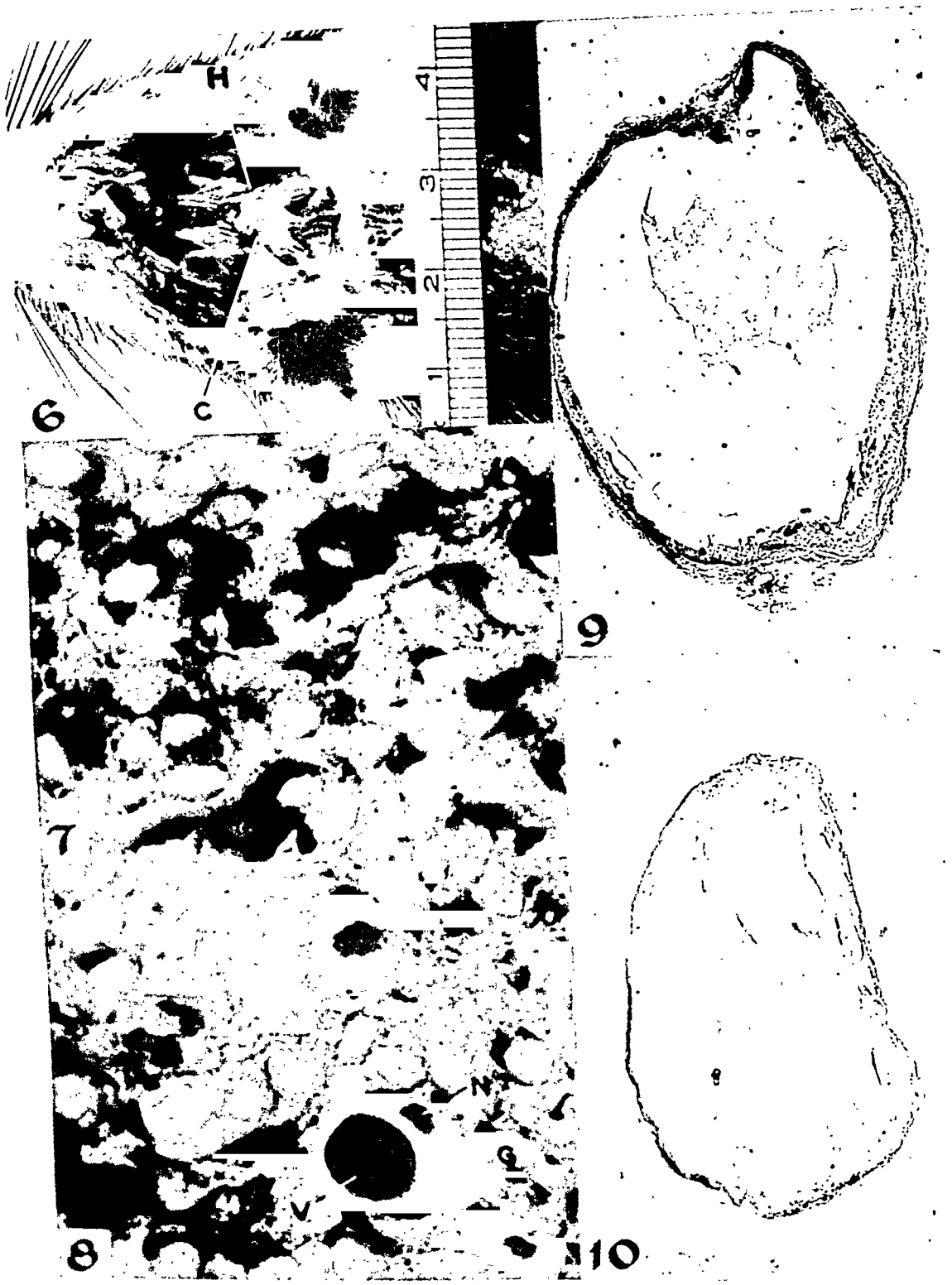

A CASE OF ATRESIA OF THE ILEUM WITH A DIVIDED KIDNEY IN THE FOETAL PIG

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ONE FIGURE

Although congenital atresia of the intestine in man has frequently been reported (Tobeck, '27; Bodon, '30; Webb and Wangensteen, '31; Cottam and Cottam, '42; Price and Chang, '46; and standard texts of human embryology), the anomaly has seldom been reported in other animals. Stamp ('43) has described a case of atresia of the ileum in the new-born calf and a case of atresia of the colon in the new-born foal, but the condition has never to our knowledge been described in the pig.

The term "intestinal atresia" is somewhat ambiguous. It may refer to cases in which the intestine is merely obstructed, to cases in which the lumen is occluded and the gut reduced in diameter, and to cases in which the intestine is completely separated into two portions, each portion ending in a blind sac. All types of atresia have been reported in man. The cases reported by Stamp are of the type in which the intestine is completely separated into two portions. The anomaly here reported is of this type also.

The anomaly was discovered in a formalin-preserved foetal pig of 25 cm crown-rump length. The duodenum and jejunum were normal. The ileum, however, gradually increased in size to a circumference of 5.1 cm a few centimeters before ending in a blind pouch (see fig. 1). Distal to the break the ileum formed another blind pouch, this time only 1 cm in circumference. From here the intestine continued to the anus with a uniform circumference of about 1 cm. Dark brown meconium

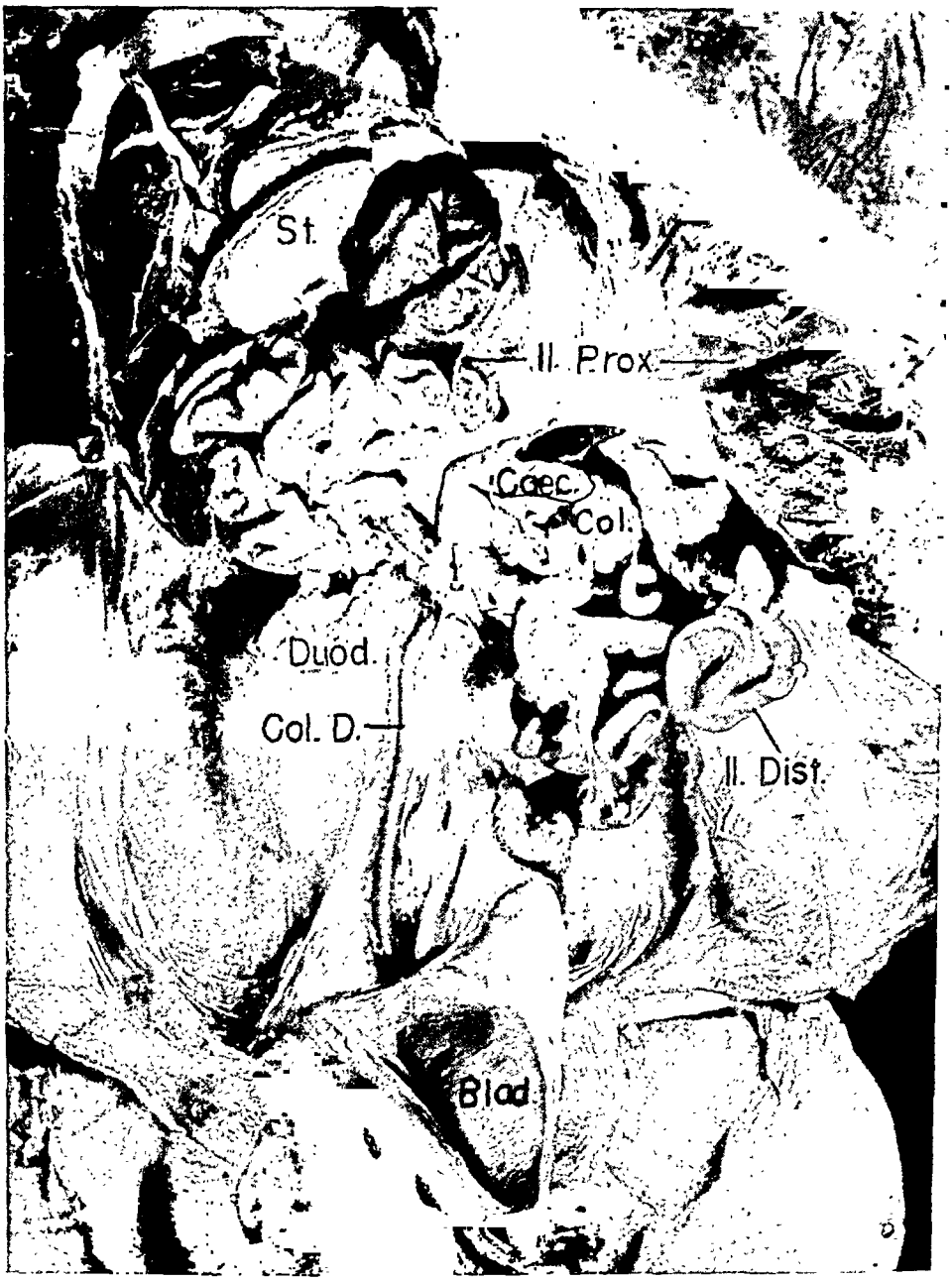

Fig. 1 A photograph of the abdominal cavity with the intestine arranged to show the two blind pouches. The lower edge of the caecum has been inked-in for greater clarity. × ¾. Blad., bladder; Caec., caecum; Col., coiled portion of the colon; Col. D., descending colon; Duod., duodenum; Il. Dist., distal portion of ileum; Il. Prox., proximal portion of ileum; Liv., liver; St., stomach.

occurred in the ileum anterior to the break. Posterior to the break the intestine was empty until a point about 3.5 cm from the ileocolic valve. Between this point and the valve there were several small bits of yellowish-brown material, and the caecum and colon were stuffed with the same material.

The mesenteric blood vessels seemed quite regular, both near the site of the atresia and along other parts of the intestine. At the point where the atresia occurred, however, a cleft extended nearly to the base of the mesentery, and the mesenteries seemed less extensive than usual at both sides of the cleft. If the broken ends of the intestine were placed end to end, a circular space was formed where the mesentery normally should have extended.

Abnormal kidneys were associated with the anomaly, but otherwise the specimen was normal. On the left side the kidney was partially constricted in two, while on the right side the kidney was entirely constricted in two. The anterior portion on the right side was shaped like a tear-drop, flattened dorso-ventrally and with its apex pointed posteriorly. The posterior portion on the same side was smaller, laterally flattened, and almost triangular when viewed from the side. The base of the triangle lay next to the urethra. These two kidney portions were connected by a common, enlarged ureter, which narrowed to normal size as it approached the bladder. The blood supply to the anterior kidney portion was normal; both renal artery and renal vein occurred in the normal position. The blood supply to the posterior portion was abnormal, however. Immediately posterior to the renal vein mentioned above, a renal vein to the posterior kidney portion arose from the post caval. This is what one would expect. The artery supplying the posterior kidney portion, on the other hand, did not originate directly from the aorta. Instead, it originated from the internal iliac artery. A situation identical to the above occurred on the left side, except that the two portions of the kidney were closer together and connected by a band of kidney tissue.

Unfortunately, case reports such as this give little clue as to the cause of the anomaly. Stamp discusses the possible causes and concludes that maldevelopment of mesenteric vessels may have caused both his cases. Our case is very similar to his and may be due to the same cause. The existence of an incomplete mesentery further suggests a deficient blood supply. It is not known whether the divided kidney is a related or an independent anomaly.

SUMMARY

A case of atresia of the ileum, in which the two portions of the intestine are completely separate, is reported. A divided kidney in the same pig is reported also.

We wish to thank Mr. Arthur E. Princehorn, Oberlin College Photographer, for taking the photograph shown in figure 1.

LITERATURE CITED

BODON, GEORG 1930 Ein Fall von fetalem Dünndarmverschluss mit Beiträgen zur Histologie und Histogenese. Virchow's Arch. Path. Anat. u. Physiol., *278*(3): 529–538.

COTTAM, GEOFFREY I. W., AND GILBERT COTTAM 1942 Congenital atresia of the duodenum, with coexisting congenital band obstruction of the lower bowel and other anomalies. J. Lancet, *62*(3): 83–85.

PRICE, SUMNER, AND THOMAS CHANG 1946 Hepatic abcess complicating atresia of the small intestine of a newborn infant. Arch. Path., *41*(4): 450–453.

STAMP, J. T. 1943 Congenital atresia of the ileum and colon. Vet. J., *99*(12): 308–310.

TOBECK, ALFRED 1927 Über angeborene Verschlüsse (Atresien) des Darmrohres. (Gleichzeitig ein Beitrag zur Frage des Entstehens der Meconium Körperchen.) Virchow's Arch. Path. Anat. u. Physiol., *265*(2): 330–353.

WEBB, C. H., AND O. H. WANGENSTEEN 1931 Congenital intestinal atresia. Am. J. Dis. Child., *41:* 262.

THE EFFECTS OF LOW PHOSPHORUS DIET AND HYPOPHYSECTOMY ON THE STRUCTURE OF COMPACT BONE AS SEEN WITH THE ELECTRON MICROSCOPE

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FOUR FIGURES

In a former paper (Barbour, '50) it has been shown that the minute structure of compact bone is made up of a consistent honeycomb-like pattern which is represented by light areas surrounded by dark walls. The light spaces seemed to be cuboidal or hexagonal in shape. More recently, R. A. Robinson reported similar findings in the abstracts of the third Conference on Metabolic Interrelations (E. C. Reifenstein, Jr., editor, '51). He has further analyzed the electron micrographs with special attention being directed toward the crystals of hydroxyapatite, which his electron micrographs are said to show. In collaboration with S. B. Hendricks and with the aid of x-ray diffraction studies, he has constructed a crystal lattice which he considers representative of the basic structure of compact bone. Robinson's method of specimen preparation involves the use of agitation and blending with a Waring Blendor and the application of high pressures with an autoclave in order to produce the thin (less than 1 μ thick) bone specimens which are needed in electron microscopy. By this means he derives micrographs which show crystal-like forms together with a few bundles of the so-called crystals.

[1] The authors wish to acknowledge with thanks the financial assistance derived from grants generously made by the Wenner-Gren Foundation for Anthropological Research, and the Committee on Research of the University of California.

The micrographs presented here (figs. 2, 3, 4) as well as those previously published (Barbour, '50) are identical with Robinson's but show the intact or bundle arrangement of the

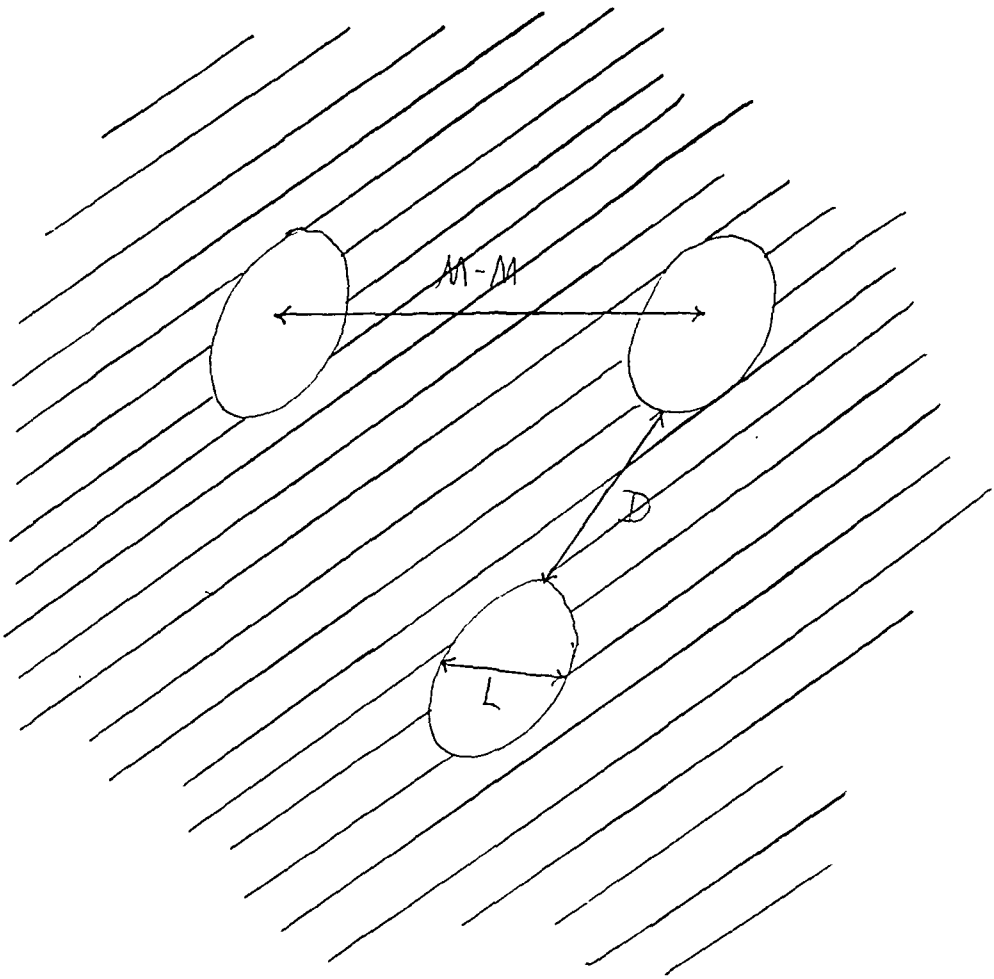

Fig. 1 Schematic representation of the submicroscopic pattern of compact bone as seen when the electron micrograph (negative) is examined under the low power of a light microscope. M–M, middle to middle measurement; D, dark area measurement; L, light area measurement.

structure which he designated as crystals. According to Schafer ('29), the smallest structures clearly visible with the ordinary optical microscope are the canaliculi (0.3 μ); the largest structures seen in the present electron micrographs are about 0.0225 μ. It becomes obvious that the minute pattern seen here

is the most basic structure yet demonstrated, or the first building block of bone as we know it in general histology. It is this pattern, then, in which pathological variations are to be sought.

Electron micrographs of compact bone[2]

The electron micrograph positive prints of compact bone which are shown in figures 2, 3, and 4 represent enlargements

Fig. 2 Electron micrograph, positive print, of compact bone taken from the femur of a one year old normal control rat. 28,000 ×.

of 28,000 times the actual specimen diameters. It is emphasized that the prints have been made from the original electron micrograph negatives which were themselves 8,000 times the actual specimen size. Under these printing conditions it is difficult to bring out the fine detail and clarity that exists on the negatives themselves. Consequently, in order to obtain an idea of the actual pattern which may represent the

[2] Detailed information on the electron microscope is well presented by Burton and Kohl ('46) and Wyckoff ('49).

basic structure of compact bone, measurements were made from the electron micrograph negatives, using the low power of an ordinary light microscope, a 6 × eyepiece, and a micrometer disc.

Fig. 3 Electron micrograph, positive print, of compact bone taken from the femur of a one year old hypophysectomized control rat. 28,000 ×.

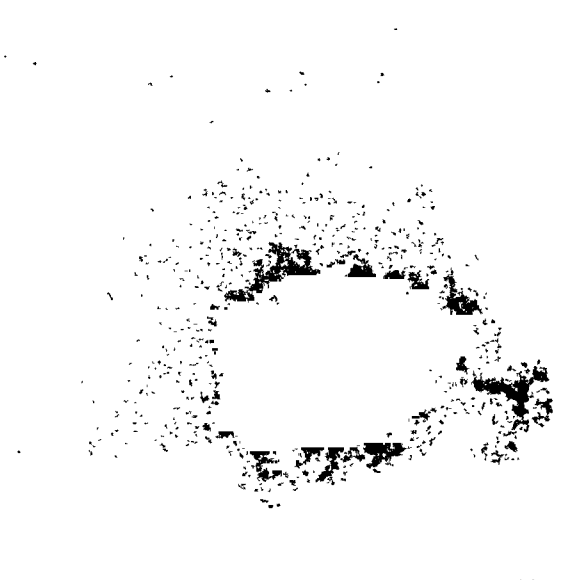

Fig. 4 Electron micrograph, positive print, of compact bone taken from the femur of a one year old growth hormone treated rat. 28,000 ×.

This procedure is considered more accurate than measuring the positive print itself. Still, it must be realized that a comparatively large error is possible. There are three reasons for this error. First, we note that the results are ob-

tained as a consequence of subjective observation and measurement. That is to say, the observer's constancy in method is depended upon for accuracy in measurement. With this factor in mind, a minimum of 30 measurements was made from each micrograph. Of each bone specimen an average of 5 micrographs was taken.

A second possible source of error lies in the choice of points for measurement. Here the observer has exercised great care in selecting random areas for this purpose. In the third place, it is well known that when any histological section is made there appear certain areas which seem to be alike yet which have different dimensions within the section. This stems from the fact that the section has probably been cut at an angle. Therefore, our level or method of cut is important. It is essential that the observer be as impartial as possible in the choice of areas to be measured and that a large number of observations be made in order to minimize these errors.

The actual measurements were of three types. The first, the middle to middle measurement (M–M) represents the distance from the middle of one light area to the middle of another. Light area measurements (L) represent the diameter or width of this particular area. Dark area measurements (D) represent the width or distance between light spaces. Extensive areas of darkness on the print (or lightness on the micrograph) are considered to be regions which are too thick to allow electron penetration. On the other hand, where areas of lightness and darkness show up as part of the pattern itself, it is reasonable to assume that there is no obstructive thickness but rather that some type of less penetrable material is present. Discussion of the possible composition of these areas may be left until later.

Care of animals

Rats of the Long-Evans strain were obtained at 21 days of age and were either hypophysectomized at this age, maintained on growth hormone, or both hypophysectomized and

given growth hormone therapy starting at 21 days. Adequate control series were also maintained. A thin wet mash of a modified McCollum's formula was used as the standard diet. All animals were injected intraperitoneally. The control animals for the hormone treated series were injected with 0.9% saline solution. The growth hormone treated animals received 12.55 mg of growth hormone over a period of 21 days. This dosage is known to have been effective (Ulrick, '52). The femurs were removed at sacrifice, cleaned, and permitted to dry without further exposure to solvents or preservatives. The hypophysectomies were performed according to a modified Smith technique ('30). The phosphorus deficient animals were maintained on a low phosphorus diet modified from Coleman et al. ('50). All normal control animals were fed *ad libitum* on the standard Green Diet 14 (Institute of Experimental Biology, University of California, Berkeley). The young control animals, however, were given the standard weanling diet from the Institute of Experimental Biology (White Diet I) in the form of a wet mash. It has been shown that newly weaned animals prosper well on this type diet. It should be noted that the animals hypophysectomized at 50 days were sacrificed only 11 days after operation. This was necessary because of the short survival time of the majority of animals in this group. It was not possible to attribute the high mortality rate to any definite physical factor.

Preparation of specimens

Specimens were prepared for electron micrographic study as follows. Each femur was air-dried after removal at autopsy and, although most of the attached periosteum and muscle had been previously removed, all bones were again cleaned by washing and external scraping. The bones were cut cross-sectionally and all marrow was removed. The shaft of each bone was cleaned internally by washing and scraping. Previously we used a method of wedge chipping of the bone in order to obtain small sections ('50). In that case, a sharp

scalpel was found to produce some sections that were thin enough for electron microscope study. In the present instance, however, we have modified this method and have found that not only more numerous and thinner sections are obtainable, but that the micrographs here are more satisfactory. The majority of these sections are thinner and permit more detail in the micrographs.

The present technique involves grinding pieces of bone which have been cut from the approximate center of the femur shaft in the cross-sectional plane. Grinding of the specimen is accomplished by use of a mortar and pestle while the specimen is immersed in a small amount of distilled water. Transfer of the specimen suspension is made from the mortar to individual clean, dry containers. Distilled water is used as a transfer medium and all containers are sealed immediately to prevent contamination. A small quantity of the suspension is pipetted onto the electron microscope screen by means of individual clean pipettes (for preparation of the screen see below). These screens are placed in separate containers, sealed, and used as soon as possible after preparation. It should be remembered, however, that all screens must be dry when placed in the electron microscope. For this reason the screens are placed on absorbent paper during suspension transfer and similarly pieces of absorbent paper are placed in each of the individual containers. Experience has shown that an excess of liquid will often harm the delicate parlodion film which covers each screen. For this reason extreme caution is observed when pipetting the specimen suspension onto the screens and precautions are taken against allowing any liquid to remain standing on the screens.

Preparation of screens

Each screen is approximately one-quarter inch in diameter and is made of a wire mesh containing about 400 meshes per square inch. It has a concave and a convex side. The screens are cleaned thoroughly by immersion in amyl acetate (stand-

ard purified solution) and then are washed repeatedly in distilled water. A 2% parlodion solution is prepared from parlodion strips and amyl acetate together with a small quantity of ether to hasten solution of the cellulose. Approximately 8 to 10 drops of this solution are placed on the surface of distilled water, which is contained in a bowl measuring about 10 inches in diameter. The parlodion spreads out over the surface of the water and creates a film which is estimated to be about 100 Å in thickness. Attention is given to the particular side of the screen which comes in contact with the film, i.e. whether it is the convex or the concave side. The technician is careful to be consistent in the side of the screen which will be covered by the film because the specimen should be placed on the film covered side of the screen. Caution exercised in this step avoids much confusion later. From two to 4 screens are usually placed side by side on the film surface. An ordinary histological slide is then placed over the screens and the surface of the film. The slide is forced down into the water, turned over while submerged, and then withdrawn. It can then be seen to support screens which are covered only on one side with the parlodion film, the other side remaining in contact with the slide. These preparations are placed in dust-free containers to dry. Each screen is used for electron microscopy as soon as possible after it has been prepared and dried. The latter precaution is taken in order to eliminate the possibility of overdrying and therefore the possibility of excess tension developing in the film. Such tension would allow the film to break more readily when exposed to the bombardment of electrons in the electron microscope. The specimen-covered screens are placed in individual containers and transported to the electron microscope.

It is expedient to exercise great care in handling all electron microscope preparations, particularly with reference to contamination. It is essential, therefore, that control slides be run in conjunction with all true specimen preparations. This procedure has been followed in all cases presented here. Blank screens were subjected to laboratory dust or contamina-

tion and were run along with the true preparations. All steps, including application of distilled water, were followed with the exception that no specimens were actually placed upon the screens. In the case of the control screens no artifacts were observed which in any way resembled pictures derived from the true specimen preparations.

RESULTS

As noted previously, electron micrographs of compact bone show a consistent honey-comb pattern made up of light electron-opaque areas surrounded by dark electron-penetrable walls.

Electron micrographs made from normal control animal material show the fundamental pattern as presented above. The consistency in size of the structures fundamental to this pattern can be seen if reference is made to the data presented in tables 1 to 4. In these tables each group of observations numbered as animal, or specimen nos. 1, 2, and 3, represents the average of 30 measurements taken from the micrograph of a separate specimen. Standard deviations ($\pm$) are also shown.

The measurements which constitute the experimental data are given in tables 1 to 4. From these measurements, the following conclusions can be drawn.

(A) The middle to middle measurement tends to increase with increasing age of the animal (tables 1, 2, and 3; particularly table 1).

(B) The measurements derived from the low phosphorus diet specimens (table 3) are interesting in the light of the data for the normal control animals of the same age (table 1). There is no significant difference in the middle to middle measurement of animals maintained on low phosphorus diet and of the normal control animals. There is, however, a distinct variation in the size of the dark areas in the phosphorus deficient specimens as opposed to the normal controls. A significant decrease in the sizes of the dark areas is noted. On the other hand, a corresponding increase in the size of the

TABLE 1

Normal control animals

Measurements presented in Angstrom units (Å)

GROUP	ANIMAL Type	NO.	A DISTANCE FROM MIDDLE TO MIDDLE OF LIGHT AREA	B WIDTH OF DARK AREA	C B AS PER CENT OF A	D DIAMETER OF LIGHT AREA	E D AS PER CENT OF A
1	21 day	1	145 ± 3.3	70 ± 2.0	48	77 ± 2.5	53
	21 day	2	133 ± 2.9	66 ± 1.6	49	67 ± 2.4	49
	21 day	3	159 ± 3.5	73 ± 2.9	46	87 ± 2.5	54
2	50 day	1	209 ± 3.5	101 ± 4.6	48	108 ± 2.9	52
	50 day	2	181 ± 4.4	80 ± 3.1	44	101 ± 3.1	56
	50 day	3	172 ± 3.8	78 ± 3.8	45	95 ± 2.5	55
3	1 year	1	212 ± 5.0	97 ± 4.1	45	115 ± 2.5	55
	1 year	2	204 ± 4.6	100 ± 3.7	49	104 ± 2.2	51
	1 year	3	200 ± 4 6	100 ± 5.0	50	100 ± 3.1	50

TABLE 2

Hypophysectomized animals

Measurements presented in Angstrom units (Å)

GROUP	ANIMAL Type	NO.	A *DISTANCE FROM* MIDDLE TO MIDDLE OF LIGHT AREA	B WIDTH OF DARK AREA	C B AS PER CENT OF A	D DIAMETER OF LIGHT AREA	E D AS PER CENT OF A
1	21 day	1	150 ± 4.8	67 ± 2.5	44	83 ± 2.5	56
	21 day	2	164 ± 4.0	78 ± 3.1	47	87 ± 2.4	53
2	50 day	1	198 ± 4.0	77 ± 2.9	39	121 ± 3.3	61
	50 day	2	200 ± 4.0	77 ± 2.5	38	123 ± 3.5	62
3	1 year	1	211 ± 6.6	100 ± 4.6	47	111 ± 3.1	53
	1 year	2	203 ± 4.8	100 ± 3.7	49	103 ± 2.4	51
	1 year	3	205 ± 5.5	96 ± 5.0	46	108 ± 3.3	54

TABLE 3

Animals on the low phosphorus diet

Measurements presented in Angstrom units (Å)

GROUP	ANIMAL / Type	NO.	A DISTANCE FROM MIDDLE TO MIDDLE OF LIGHT AREA	B WIDTH OF DARK AREA	C B AS PER CENT OF A	D DIAMETER OF LIGHT AREA	E D AS PER CENT OF A
1	21 day	1	151 ± 3.1	56 ± 1.8	37	94 ± 2.4	63
	21 day	2	125 ± 2.4	48 ± 2.2	31	80 ± 2.9	69
	21 day	3	147 ± 4.4	58 ± 2.7	39	88 ± 3.5	61
2	50 day	1	196 ± 3.1	80 ± 3.3	40	118 ± 2.2	60
	50 day	2	212 ± 2.5	87 ± 2.5	41	123 ± 2.5	59
3	1 year	1	210 ± 5.3	83 ± 4.8	39	125 ± 2.7	61
	1 year	2	193 ± 5.5	62 ± 3.3	32	130 ± 3.1	68

TABLE 4

Hypophysectomized control, hypophysectomized plus growth hormone, normal control, and normal animals plus growth hormone.

All animals 21 days at instigation of therapy

Measurements presented in Angstrom units (Å)

GROUP	ANIMAL / Type	NO.	A DISTANCE FROM MIDDLE TO MIDDLE OF LIGHT AREA	B WIDTH OF DARK AREA	C B AS PER CENT OF A	D DIAMETER OF LIGHT AREA	E D AS PER CENT OF A
1	H control	1	209 ± 5.6	98 ± 4.0	46	111 ± 3.0	53
	H control	2	209 ± 4.7	93 ± 3.8	44	116 ± 2.9	56
	H control	3	209 ± 5.0	98 ± 5.1	46	111 ± 5.8	53
	H + growth hormone	1	231 ± 4.4	106 ± 2.5	46	124 ± 2.4	54
	H + growth hormone	2	221 ± 5.1	98 ± 3.8	44	123 ± 2.7	56
	H + growth hormone	3	229 ± 5.3	108 ± 4.1	47	120 ± 2.0	53
	Normal control	1	219 ± 4.1	104 ± 2.7	47	115 ± 2.4	53
	Normal control	2	217 ± 5.3	99 ± 3.8	46	118 ± 3.7	54
	Normal control	3	231 ± 5.1	109 ± 2.7	43	122 ± 2.2	55
	Normal + growth hormone	1	216 ± 6.6	97 ± 5.0	45	118 ± 2.9	54
	Normal + growth hormone	2	222 ± 6.1	101 ± 4.2	45	120 ± 2.5	54

light areas is seen. The middle to middle measurements again seem to be related to the age of the animal, increasing with increasing age.

(C) The data in table 4 indicate a possible decrease in the middle to middle measurements of hypophysectomized animals, group 1, over those of the normal control animals of this series, group 3. Such a suggestion was not verified in later studies (table 2). There was no significant variation in the measurements taken from hypophysectomized controls, normal controls, normal animals on growth hormone therapy, or in hypophysectomized animals on growth hormone therapy.

DISCUSSION

The structures visible in electron micrographs of compact bone range in size from 0.0048 μ to 0.0212 μ. This is far below the range of structures visible in histological sections under the light microscope. The fine canaliculi are among the smallest elements seen in bone under the ordinary light microscope and these are about 0.3 μ in diameter.

It seems reasonable to assume that the dark areas seen in the submicroscopic pattern represent the inorganic constituents of bone. There are two reasons for such an assumption. First, we note that the dark areas are continuous throughout the bone pattern, whereas the light spaces are discontinuous. Since bone is such a hard supporting structure, it seems logical that its frame should consist of rigid inorganic material rather than semi-liquid discontinuous organic elements (Ascinzi, '50; and Clark, '31). Secondly, a very significant decrease in the dimensions of the dark areas is noted when there is a dietary deficiency of phosphorus. This deficiency probably causes a diminution in the parts of the bone which are inorganic in character.

If the dark areas are composed of inorganic material, it seems reasonable to assume that the light areas are concerned in some way with the organic components of bone. They may actually represent organic material or they may be cavities which at one time contained organic material.

Age studies indicate that there is an increase in the middle to middle values with age. This means that there is a corresponding increase in the amount of both dark and light material with age. That is, all the measurements made from the older animal micrographs are consistently greater than those taken from the young animal micrographs. The amount of dark and light material, however, remains constant relative to the middle to middle measurement. This may indicate that the growth or maturation of bone involves an addition of both organic and inorganic constituents in the submicroscopic structure even throughout maturity (Strobino and Farr, '49; or Dawson, '21). Hence, bone which has been formed early in life may be modified as the animal ages, not only structurally as seen histologically but also continuously at the molecular level. Such an hypothesis emphasizes the concept of a dynamic equilibrium existing in bone even in its most fundamental mineral structure. This is corroborated by Logan and Lewis ('39).

Growth hormone therapy in rats causes an increase in the overall length and weight of long bones as well as elevated body protein nitrogen and phosphorus levels. When the structures visible in electron micrographs of compact bone are measured, however, there is no significant difference in the values derived from normal-growth hormone treated animals and those from the normal controls. Since this hormone dosage is known to have been effective in the body generally, we assume that growth hormone has no effect upon the submicroscopic structure of bone.

Electron micrographic studies were performed on hypophysectomized-growth hormone treated animals. There was a slight increase in the middle to middle measurements of hypophysectomized-growth hormone treated animals as compared with the normal-growth hormone treated animals. This difference was not great enough, however, to allow a definite conclusion and the need for more extensive study is indicated.

In conclusion, it is evident that whereas both hypophysectomy and administration of growth hormone induces clear effects with respect to the overall increase in size of bones, neither causes a significant change in the submicroscopic structure.

The electron micrographic picture derived from animals on an extremely low phosphorus diet shows that there is a pronounced decrease in the amount of dark material in the bone. We assume that the dark areas represent inorganic material, as previously discussed. Therefore, the effect of phosphorus deficiency is to reduce the amount of inorganic material present even in the submicroscopic pattern. These micrographs show a corresponding increase in the dimensions of the light areas. A reciprocal relationship is evident.

In order to substantiate the assumption that even the fundamental pattern is affected by phosphorus deficiency, we must consider the two effects of this deficiency. The first is to reduce the mineral intake of the animal to a point where there is not sufficient material present to supply the needs of the forming bone, therefore, the new presumptive bone remains unmineralized and is called osteoid. In other words, the tissue itself is formed just as bone is formed except that little or no mineral is deposited in it. If the bone sections used in the present investigation were actually derived from areas where osteoid was paramount, it seems evident that the fundamental pattern would show considerable reduction in the dark areas (as above). The second effect of an extremely low phosphorus diet is to cause demineralization of preformed bone. This occurs in order to provide mineral elements for the support of growth and proper function in the soft body tissues. If such a process of demineralization is characteristic of the whole bone, it is readily apparent that the fundamental structure would itself be altered. The electron micrographic picture, therefore, is a result of either one or both of these processes. Young bone changes may be most reasonably considered dependent upon the formation of osteoid. Older bone

changes are probably more easily related to the process of demineralization which is seen in phosphorus deficiency.

SUMMARY

1. Electron micrographs were made from thin chips of compact bone. The specimens studied were: (1) normal control, hypophysectomized, and low phosphorus diet animals from three age groups — young, young adult, and old adult animals; (2) normal controls, hypophysectomized-normal, hypophysectomized-growth hormone treated, and normal-growth hormone treated animals.

2. All specimens show a similar and consistent pattern which resembles a honeycomb with light spaces surrounded by dark walls. The light spaces seem to be round or cuboidal in shape. This pattern is considered to represent the fundamental structure of compact bone.

3. The dark areas probably represent the inorganic components of compact bone.

4. Bone materials taken from animals maintained on an extremely low phosphorus diet show a distinct reduction in the presumptive inorganic components of the fundamental pattern.

5. Growth hormone seems to have no effect upon the submicroscopic pattern of bone.

6. Hypophysectomy does not influence the fundamental pattern of bone significantly.

7. Age studies show that there is a distinct size change in the submicroscopic pattern of animal bone with time. This indicates that the growth or maturation of bone may involve a long continued increase in the bone constituents even in this fundamental structure.

LITERATURE CITED

ASCENZI, A. 1950 On the existence of bonds between ossein and inorganic bone fraction. Science, *112:* 84.

BARROW, E. P. 1950 A study of the structure of fresh and fossil human bone by means of the electron microscope. Am. J. Phys. Anthrop., *8:* 3, 315.

BURTON, E. F., AND W. H. KOHL 1946 The Electron Microscope. 2nd ed. N.Y., Reinhold Pub. Co.

CLARK, J. H. 1931 A study of tendons, bones, and other forms of connective tissue by means of x-ray diffraction patterns. Am. J. Physiol., *98:* 328.

COLEMAN, R. A., H. BECKS, F. VAN N. KOHL AND D. H. COPP 1950 Skeletal changes in severe phosphorus deficiency of the rat. Arch. of Path., *50:* 209.

DAWSON, A. B. 1921 The age order of epiphyseal union in the long bones of the albino rat. Anat. Rec., *31:* 1.

LOGAN, M. A., AND W. K. LEWIS 1939 Solubility of bone salt. IV. Solubility of bone in biological fluids. J. Biol. Chem., *127:* 705.

REIFENSTEIN, E. C., JR., EDITOR 1951 Third Conference on Metabolic Interrelations, Josiah Macy, Jr., Foundation, Progress Assn., Inc., N.J., 271–289.

SCHAFER, E. S. 1929 Essentials of Histology. 12th ed. Philadelphia, Longmans, Green and Co.

SMITH, P. E. 1930 Hypophysectomy and replacement therapy in the rat. Am. J. Anat., *45:* 205.

STROBINO, L. J., AND L. E. FARR 1949 The relation to age and function of regional variations in the nitrogen and ash content of bovine bone. J. Biol. Chem., *178:* 599.

ULRICK, F. 1952 Dissertation in partial satisfaction of the requirements for the degree of Doctor of Philosophy, University of California.

WYCKOFF, R. W. G. 1949 Electron Microscopy; techniques and applications. N.Y., Interscience Pub. Co.

TESTICULAR DEVELOPMENT IN THE RHESUS MONKEY[1]

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TWENTY-EIGHT FIGURES

An analysis of normal testicular development in the rhesus monkey, *Macaca mulatta,* was undertaken in order to provide basic data for the interpretation of changes brought about experimentally by the action of gonadotrophic hormones in the immature animal. An obstacle to the effective use of the monkey for this purpose has been the lack of exact knowledge of the developmental status of the testes of animals of known ages, so necessary as a foundation for any experimental work. Even in isolated experiments, normal control animals of the same age raised under the same conditions at the same time are rarely available for the monkey. No colony is large enough to supply such paired animals in sufficient numbers. Rarely is it possible even to obtain animals of known age, while littermates are out of the question since no male twins have thus far been reported. Purchased animals, which are usually of unknown history, although of the same body weight and approximate appearance, may be very different in age and sexual development. It is true that certain observations help

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TABLE 1

Development of testis of the monkey, Macaca mulatta. Sequence of changes correlated with age

AGE OF MONKEY	TESTIS LENGTH	SEMINIFEROUS TUBULE DIAMETER	TESTICULAR DIFFERENTIATION
	cm	*micra*	
Fetal life: 72 d.	0.04	70– 80	Few spermatogonia present; Sertoli nuclei basal; Leydig cells differentiated.
154 d.	0.06		
Birth to 3–4 mo.	0.75	50– 60	Regression of Leydig and Sertoli cell differentiation; Sertoli nuclei no longer basal.
8 mo. to 1 yr. 3 mo.	1.10	50– 60	Increase in spermatogonia.
1 yr. 3 mo.–2 yr. 7 mo.	1.10–1.60	50– 60	*Relatively little advance.*
ca. 2 yr. 7 mo.	2.0	50– 60	Leydig cells epithelioid, not mature; Sertoli cells increase in number.
ca. 2 yr. 9 mo.	2.0	70–100	Sertoli nuclei move basally; occasional spermatocytes appear.
2 yr. 10 mo.–3 yr. 2 mo.	2.2	100–150	Spermatids appear and a few differentiate to sperm; Leydig cells mature.
3 yr.–3 yr. 5 mo.	3.00	150–200	Sperm; tubules filled with debris.
3 yr. 5 mo.–4 yr.	3.25	200–250	Spermatogenesis complete. Tubules orderly and free of debris.
from 4 yr.	4.00	250–300	Fully active.

in establishing the probable age. Body weight and body length (sitting height) may be compared with known growth curves, and roentgenograms with the normal standards of skeletal differentiation. Dental age is somewhat more easily determined and applied (Hurme and van Wagenen, '53). However, both dental and osseous development are of value only when significant steps in differentiation coincide with those parts of the life span which are of interest in a particular experiment.

It remained essential for the work with gonadotrophins to establish the age at which critical changes occur in the testis, and the sequence and duration of each developmental phase. Material suited for this study has accumulated since 1935 from the monkey colony established in the Department of Obstetrics at Yale University. The colony is an inbred group of rhesus monkeys *(Macaca mulatta)* now in its sixth generation. Histological preparations from biopsies and autopsies were studied from 40 male monkeys of known age, from fetal life to eleven years. Table 1 gives in outline form the critical periods in growth and differentiation of the testes.

Figure 1 (described below) is based on serial observations of body weight and testis length of animals born and raised in the colony. Of a total of 24 normal untreated animals studied during the first year, 15 were followed through the second year, 14 through the third year and 12 to the end of the fourth year. The smaller numbers in succeeding years means only that these normal monkeys were allocated to experiments.

OBSERVATIONS

Fetal development. The testicular tubules before birth are still short, are only slightly coiled and are separated by much interstitial tissue. However, both tubular and intertubular tissue show evidence of early differentiation. The degree of development exceeds that attained for months or even years after birth, being almost comparable to that of the preadolescent testis. At 90 to 110 days of fetal life the tubules are widely separated by abundant interstitial tissue (figs. 2,

19 and 20). This tissue consists for the most part of small epithelioid cells, which, though not so large as in the adult, are recognizable as Leydig cells. The tubules of the testes at this time are short and only slightly coiled but are larger in diameter (70–80 μ) than after birth. The Sertoli cells have acquired morphological features which are here interpreted as differentiation. The cytoplasm is abundant and filamentous and extend across the lumen; the nuclei occupy a peripheral or basal position in the cytoplasm.

The presence of abundant interstitial tissue (identified as Leydig cells) has been described in fetal testes of both the human and the horse (Bouin and Ancel, '03; Cole et al., '33; Gillman, '48) and has been correlated with the increased amounts of gonadotrophin present in the maternal blood of these species. Since chorionic gonadotrophin has been reported in the urine of the pregnant monkey (Hamlett, '37), it is reasonable to attribute to this agent the Leydig cell growth and differentiation in the fetal monkey testis.[2] The descended testis characteristic at birth of both man and monkey has also been correlated with intrauterine action of gonadotrophin (Engle, '32b; Wislocki, '33).

Post-natal regression. Actual regression in the development of the testes occurs after birth (figs. 3 and 21). At birth the Sertoli cell differentiation is still present and nuclei are basal. The interstitial cells have decreased in size and probably in number. Figure 21 shows, especially by comparison with the fetal picture in figure 20, the more closely packed tubules at birth. This regression is most pronounced within the first 3 months and resumption of development may not become evident before 9 months to 1 year 3 months (figs. 4, 5, 6). The diameter of the seminiferous tubules decreases to 50 μ and does not increase appreciably during the first year, still measuring between 50 to 60 μ at 1 year 3 months (fig. 7).

[2] Evidence will be given in a paper devoted to the effects of interstitial cell stimulating gonadotrophins in the prepuberal male monkey that differentiation in the Sertoli cells is also due to the chorionic gonadotrophin. Engle ('32) shows this change in his illustrations.

The tubules grow in length, however, and become more convoluted, accounting for the increasing size of the testis. The Sertoli cells lose the cytoplasmic differentiation attained during intrauterine life, and the nuclei of these cells again come to fill the lumen of the tubules. These and a relatively few basally situated spermatogonia are the only cells present in the tubule. Differentiated Leydig cells disappear. Within 3 or 4 months (figs. 3, 4 and 5) after birth, the intertubular spaces have become narrow and the tubules are closely packed together. The only intertubular cells which remain, except for a few flattened endothelial cells, are inactive Leydig or capsule cells with small dark staining nuclei, which lie very close together and surround the tubules in 1 or 2 concentric layers.

Beginning differentiation. The regression in differentiation of tubular and intertubular tissue may be attributed to the withdrawal at birth of maternal hormone influences. There supervenes a period which lasts through most of the first year, during which tubules are not elaborated cytologically, though they lengthen and become more convoluted. The tubules at this time characteristically contain only Sertoli cells and scattered spermatogonia. Intertubular tissue is scanty and Leydig cells are not distinguishable. Early in the second year spermatogonia become more numerous, forming a continuous basal layer in some tubules. The multiplication of spermatogonia sometimes becomes obvious at 11 months, and is more evident still between 1 year 3 months, and 1 year 9 months (figs. 7 and 8). These cells are larger, more rounded and possess more abundant cytoplasm than the previously seen primitive sex cells.

Although the tubules had begun to increase slightly in size by 2 years 2 months to 2 years 4 months, they still measured between 50 and 60 μ, and even by 2 years 7 months they reached only 70 to 80 μ, corresponding to the diameter in late fetal life (compare fig. 3 with figs. 9, 15 and 16). It is more difficult to assess the increase in tubule length which un-

doubtedly occurs. Some indication of this is the gradual overall increase in testis size (table 1 and figure 1).

For an entire year, extending through most of the third year of the individual's life, no appreciable further advancement in differentiation of the germinal elements of the tubules takes place (compare figs. 8, 9 and 11, 12). The next phases in development concern the Sertoli and the Leydig cells.

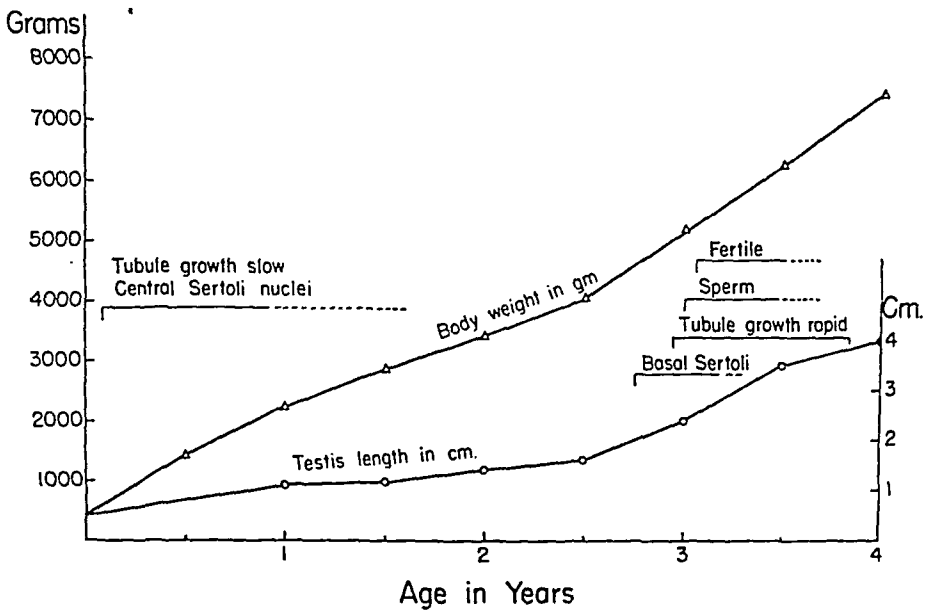

Fig. 1 Testis length correlated with body weight and age of normal control rhesus monkeys from the colony at Yale University School of Medicine. The serial observations were based on twenty-four animals through the first year. Allocation to experiments reduced this number to twelve at end of the fourth year. The histological observations were based on serial biopsy and autopsy of 40 male monkeys of known age.

Differentiation of Sertoli cells. At 2 years 7 months, when the tubules have reached 70 to 80 μ in diameter, multiplication with differentiation, of Sertoli cells occurs. The increase of Sertoli nuclei may be very conspicuous. The enlarging tubules are filled with these nuclei before the differentiation becomes pronounced (fig. 9). The nuclei enlarge and become pear-shaped with indentations, the chromatin is scattered, and there are one or more prominent nucleoli. The cytoplasm increases in amount, becomes filamentous and stretches across

to fill the lumen of the tubule. The nuclei come to lie more and more basally (figs. 10, 13, 15 and 16).

Differentiation of Leydig cells. Accompanying the differentiation of Sertoli cells, between 2 years 7 months and 2 years 9 months, the tubules become less crowded within the testis. The vascularity of the intertubular tissue is increased and the tissue fluid increases. At this time, differentiation of interstitial cells occurs. They enlarge and become rounded (epithelioid). Although not fully mature, at 2 years 9 months, they are now definitely recognizable as Leydig cells (fig. 10), apparently differentiated from cells hitherto arranged in layers around the tubules. Proliferation from encapsulating cells can be seen in illustrations from the fetal testis (fig. 20). This subject is further developed and illustrated by Simpson and van Wagenen ('53). The small, elongated, dark-staining nuclei of capsule cells enlarge and become rounded; the chromatin scatters and the nucleus therefore becomes light-staining. One or sometimes two prominent nucleoli appear. Abundant vacuolation in the peripheral part of the cytoplasm is characteristic of the Leydig cells of the mature testis but is not commonly seen in animals that have recently matured. The number of Leydig cells recognizable by these criteria in normal mature animals is surprisingly small; they form clumps usually containing no more than 2 to 5 cells and rarely 10 to 20.

Differentiation of germinal elements of the tubules. The appearance of *primary spermatocytes* marks the onset of activity in the germinal elements of the tubule which will lead to sperm formation. Between 2 years 7 months and 2 years 9 months (figs. 9 and 10) the tubule diameter has increased to about 100 μ. A few primary spermatocytes, distinguishable by their size, position and distribution of chromatin in the spireme, appear in the meshes of the Sertoli cell cytoplasm. No open lumen is present in the tubule at this time. The first formed spermatocytes undoubtedly desquamate, or degenerate, and their number does not increase immediately (figs. 13, 15, 16). Only a few scattered primary

spermatocytes were found in tubules of four animals between 2 years 9 months and 2 years 10 months of age. After 2 years 10 months, spermatocytes begin to accumulate in great numbers in the tubules and succeeding stages of development then follow rapidly (figs. 14, 17).

A series of four biopsies from the testes of Mm 642 illustrates the intratubular development from the quiescent prepuberal state to the formation of spermatocytes (figs. 11 to 14). Adequate material is not available to support general statements. However, it is seen in this animal that 4 months elapsed between the assumption of a basal position by the Sertoli nuclei and the formation of the first spermatocytes. Three weeks later the number of spermatocytes had increased but no further differentiation of cell types had occurred. In a second series of biopsies (figs. 15 to 18) from another maturing animal, Mm 282, the first testis sample was taken after migration of the Sertoli nuclei and the accompanying tubule enlargement; the last sample was taken when fully formed sperm were present. The entire period covered 10 months. At the end of the first $4\frac{1}{2}$ months the only advance to be seen was an occasional germinal cell, lying central to the Sertoli cell layer, in which a spireme was forming. The final organization of the seminiferous tubule with clearing of the lumen is shown in 3 stages for the monkey Mm 686 in figures 23 to 25, and the age span covered was from 2 years 10 months to 3 years 5 months.

Spermatids and sperm were first seen in the testicular tubules at 2 years 10 months, but in other animals this degree of development was not noted until 3 years or 3 years 6 months of age. Spermatids appeared when the tubules had attained a diameter of 100 to 150 μ. A considerable time appears to be consumed in the final ripening to form the sperm. The slender sperm tails, even after they have become elongated, remain for a time difficult to stain. During this terminal ripening phase, from the end of the third year to the middle of the fourth, desquamation of cells in all the later stages of development is occurring and the tubule lumen is packed

with debris (figs. 23 to 25). By the time the tubules are opened and cleared of all debris the diameter of the tubules has increased to 200 μ and the cells of the stratified epithelium are arranged in more orderly fashion (figs. 26 to 28). Fully mature tubules at the height of activity measure 250 to 300 μ.

Lacking monkeys of known ages, experimentalists heretofore have classified males simply as immature or mature. Body weight has been the useful index in such classifications, and where the reports have been accompanied by illustrations of the testes, the histology and body weights have agreed well with the data here presented (Engle, '32a; Smith, '38 and '44). Hartman ('32), who maintained males of known age, reported sexual maturity as occurring within the upper limits here assigned. Figure 1 shows the correlation between body weight, testis size and age of the monkeys used in this study. In all instances the measurements of the testes were found to give a reliable index of the tubular diameter and differentiation, and such measurements, correlated with the finding summarized in table 1 and figure 1, are useful in selecting male monkeys for experimental work with gonadotrophins.

DISCUSSION

Testicular development during childhood is described to be as slow as that observed here for comparable periods in the monkey, an interval of 3 years in the monkey being considered roughly equivalent to 15 years in the human. The diameter of the testicular tubules is surprisingly similar. Charny et al. ('52) give the following measurements from a developmental series (mostly from biopsies) in the human: tubule diameter 66 μ from 3½ weeks to 4 years; an increase only to 72 μ at 10 years and 85 μ at 12 years; an increase to 100–150 μ at puberty, and an average adult measurement of 150–180 μ. (Compare measurements for the monkey as given in table 1.)

The time when dedifferentiation or regression of Leydig tissue occurs in the human has not been ascertained accu-

rately but it is generally assumed that the hypertrophy of the fetal period, being dependent on maternal hormones, probably disappears soon after birth. Albert ('52), though finding it somewhat variable and uncertain, assumes that regression may occur within a week after birth. Charny et al. ('52) find no Leydig cells distinguishable 3½ weeks after birth.

Theoretically one might expect that at puberty differentiation, or even hypertrophy of Leydig cells would precede differentiation of the tubules, judged from the ability of testosterone to stimulate spermatogenesis and the supposed production of male hormone by Leydig cells. In this study, Leydig cells were found to be distinguishable from indifferent intertubular cells at about the same period as the earliest differentiation occurred in the tubules. The appearance of the fully functional cells (on basis of size, rounded cell body and granular or vacuolated cytoplasm) was not seen, however, until spermatogenesis was far advanced. Albert et al. ('52) in a recent review of the developmental stages in the human testis have stressed the fact that the changes leading to maturity at puberty definitely occur in the tubules before they do in the Leydig cells. Sniffen ('52) also describes the appearance of the first spermatocytes in human testes, e.g. at 13 years of age, before differentiation of the Leydig cell has occurred. To quote: "In early puberty, when the secondary sex characteristics are beginning to develop and tubular activity is well established, the interstitial cells seem to lag behind and remain undifferentiated." When they appeared during the next few years, however, say by 17 years, by differentiation from mesenchymal cells, more Leydig cells were observed than were characteristic in the adult. Hooker ('44) also reports that in the bull the differentiation of the tubule occurs earlier than that of the Leydig cells. He states, "No striking change in either the Leydig cells or the androgen content of the testes was evident at puberty which apparently occurred during the second 6 months of life. The great changes in androgen content after 2 years of age were ac-

companied by changes of comparable degree in vacuolation and numbers of Leydig cells."

It may be noted that in descriptions of the development of the human testis by Charny et al. ('52), Albert et al. ('52) and Sniffen ('53), these workers are conservative about the differentiation of the spermatogonia from Sertoli cells in the testis early in childhood, referring to the presence of a few sex cells and a synctium. In the description given here of the development of the testes of the monkey the enlarged, rounded, basally located cells seen from birth onward have been designated spermatogonia. Sertoli cells have been described, rather than designating a syncytium, though it is realized that the boundary of individual Sertoli cells is difficult to distinguish either in the immature or the mature testis. The opening of a lumen in the seminiferous tubule also is described here as occurring relatively later than it is given in the accounts of human development; the potential lumen is considered to be filled successively by the nuclei of the Sertoli cells, then by their filamentous cytoplasm, and later by successive generations of desquamating unripe and finally ripe germinal elements, before a truly patent lumen is established in the adult.

SUMMARY

Fetal life. During late fetal life the testicular tubules of the rhesus monkey attain a diameter of 70 to 80 μ and reach a stage of development not seen again until prepuberal changes appear. Although only Sertoli cells and a few spermatogonia are present, the nuclei and cytoplasm of the former show differentiation. The nuclei come to lie near the basement membrane and the cytoplasm fills the lumen. Interstitial cells are abundant, filling the wide intertubular spaces and many are differentiated and identifiable as Leydig cells.

Post-natal regression and subsequent slow growth. Definite regression of the testes occurs after birth. The tubules decrease to a diameter of 50 to 60 μ and remain so for the first year while increasing slowly in length. The interstitial cells decrease in number, dedifferentiate, and are not again clearly

distinguishable as Leydig cells until late in the third year. Only a few scattered spermatogonia are seen in the post-natal period, but they become more numerous, appearing enlarged and rounded at the end of the first year.

Adolescence and maturity. After this prolonged period of slow development, lasting until the end of the third year, changes which lead to maturity follow in rapid succession. Leydig cells differentiate. Sertoli cells increase in number and become differentiated, with filamentous cytoplasm filling the lumen of the enlarging tubules and with nuclei lying basally. Primary spermatocytes appear and the changes leading to formation of spermatozoa take place rapidly. The earliest appearance of spermatozoa was observed at 2 years 11 months, the latest at 3 years 5 months.

LITERATURE CITED

ALBERT, A., L. O. UNDERDAHL, L. F. GREENE AND N. LORENZ 1953 Male hypogonadism: I. The normal testis. Proc. Staff Meetings Mayo Clinic *28:* 409–422.

BOUIN, P., AND P. ANCEL 1903 Recherches sur les cellules interstitielles du testicule des mammifères. Arch. de zool. expér. et gén., *1:* 437–523.

CHARNY, C. W., A. S. CONSTON AND D. R. MERANZE 1952 Testicular developmental histology. Annals N. Y. Acad. Sci., *55:* 597–608.

COLE, H. H., G. H. HART, W. R. LYONS AND H. R. CATCHPOLE 1933 The development and hormonal content of fetal horse gonads. Anat. Rec., *56:* 275–293.

ENGLE, E. T. 1932a The action of extracts of anterior pituitary and of pregnancy urine on the testis of immature rats and monkeys. Endocrinology, *16:* 506–512.

———— 1932b Experimentally induced descent of the testis in the macacus monkey by hormones from the anterior pituitary and pregnancy urine. Endocrinology, *16:* 513–520.

GILLMAN, J. 1948 The development of the gonads in man, with a consideration of the role of fetal endocrines and the histogenesis of ovarian tumors. Carnegie Inst. Wash., Contrib. to Embryol., *32:* 81–131.

HAMLETT, G. W. D. 1937 Positive Friedman tests in the pregnant rhesus monkey, *Macaca mulatta.* Am. J. Physiol., *118:* 664–666.

HARTMAN, C. G. 1933 Cited by Schultz *in* The Anatomy of the Rhesus Monkey Ch. II, Growth and Development. Edited by C. G. Hartman and W. L. Straus, Jr. The Williams and Wilkins Co., Baltimore.

HOOKER, C. W. 1944 The postnatal history and function of the interstitial cells of the testis of the bull. Am. J. Anat., *74:* 1–37.

HURME, V. O., AND G. VAN WAGENEN 1953 Basic data on the emergence of deciduous teeth in the monkey *(Macaca mulatta)*. Proc. Am. Phil Soc., *97:* 291–315.

SIMPSON, M. E., AND G. VAN WAGENEN 1953 Persistent nodules in testis of the monkey associated with Leydig cell hyperplasia induced by gonadotrophins. Cancer Research (in press).

SMITH, P. E. 1938 Comparative effects of hypophysectomy and therapy on the testes of monkeys and rats, *in* Les Hormones Sexuelles, Edit. L. Brouha, Hermann et Cie., Paris.

———— 1944 Maintenance and restoration of spermatogenesis in hypophysectomized rhesus monkeys by androgen administration. Yale J. Biol. Med., *17:* 281–287.

SNIFFEN, R. C. 1952 Histology of the normal and abnormal testis at puberty. Annals N. Y. Acad. Sci., *55:* 609–618.

WISLOCKI, G. B. 1933 Observations on the descent of the testis in the macaque and in the chimpanzee. Anat. Rec., *57:* 133–148.

PLATE 1

EXPLANATION OF FIGURES

Figs. 2 to 10 *Developmental stages* in Macaque testes, from biopsies and autopsies. H.E. × 237.

2 Testis of Mm *486*, 96 day fetus. Tubules are short and straight, diameter 60–70 μ. Only Sertoli cells and a few spermatogonia are present in tubules. Sertoli cells are large and fill the lumen. Intertubular spaces are wide and contain many cells, some epithelioid.

3 Testis of Mm *601* at birth (174 da. gestation). Testis biopsy. Tubule diameter 60–80 μ. Nuclei of Sertoli cells are basal and cytoplasmic strands fill tubule lumen. Spermatogonia are sparse. Intertubular tissue is abundant but undifferentiated.

4 Testis of Mm *630* at 3 mo. More coiling of tubules is present; diameter 50–60 μ. Sertoli cytoplasm is developed and still fills the lumen. A few spermatogonia are present. Intertubular spaces are narrow and interstitial cells have regressed.

5 Testis of Mm *668* at 3 mo. 25 da. Considerable growth in length with coiling of tubules has occurred. Tubules are small, 50–60 μ, compact, filled with Sertoli nuclei. There are occasional spermatogonia. The peritubular arrangement of dark stained nuclei of intertubular tissue is clearly seen.

6 Testis of Mm *619* at 4 mo. 24 da. Tubules are small and closely packed. The size has not changed, 50–60 μ. Sertoli nuclei fill the lumen, only occasional spermatogonia being seen.

7 Testis of Mm *550* at 1 yr. 3 mo. 24 da. Tubules are still small, diameter 40–50 μ. Spermatogonia are now increased in number and size. Nuclei of undifferentiated cells fill the lumen. Only dark stained nuclei in rows around tubules are seen in narrow peritubular spaces.

8 Testis of Mm *385* at 1 yr. 8 mo. 7 da. Tubules are still small (diameter 50 μ). Sertoli nuclei continue to crowd the lumen. Intertubular tissue is undifferentiated.

9 Testis of Mm *510* at 2 yr. 7 mo. 6 da. Note that during an entire year there has been no appreciable advance in development except in multiplication of Sertoli cell nuclei and a slight increase in diameter of tubule (60–70 μ). Some of the interstitial cells are now lighter staining.

10 Testis of Mm *665* at 2 yr. 9 mo. 21 da. Tubules are now definitely larger (90 μ). Sertoli cells have moved to the periphery of the tubule. Spermatogonia are numerous and rounded. Rounded Leydig cells are now frequently seen.

FOLLICULAR DEVELOPMENT IN MONKEY

H. HOUSTON MERRITT AND C. E. SIMPSON

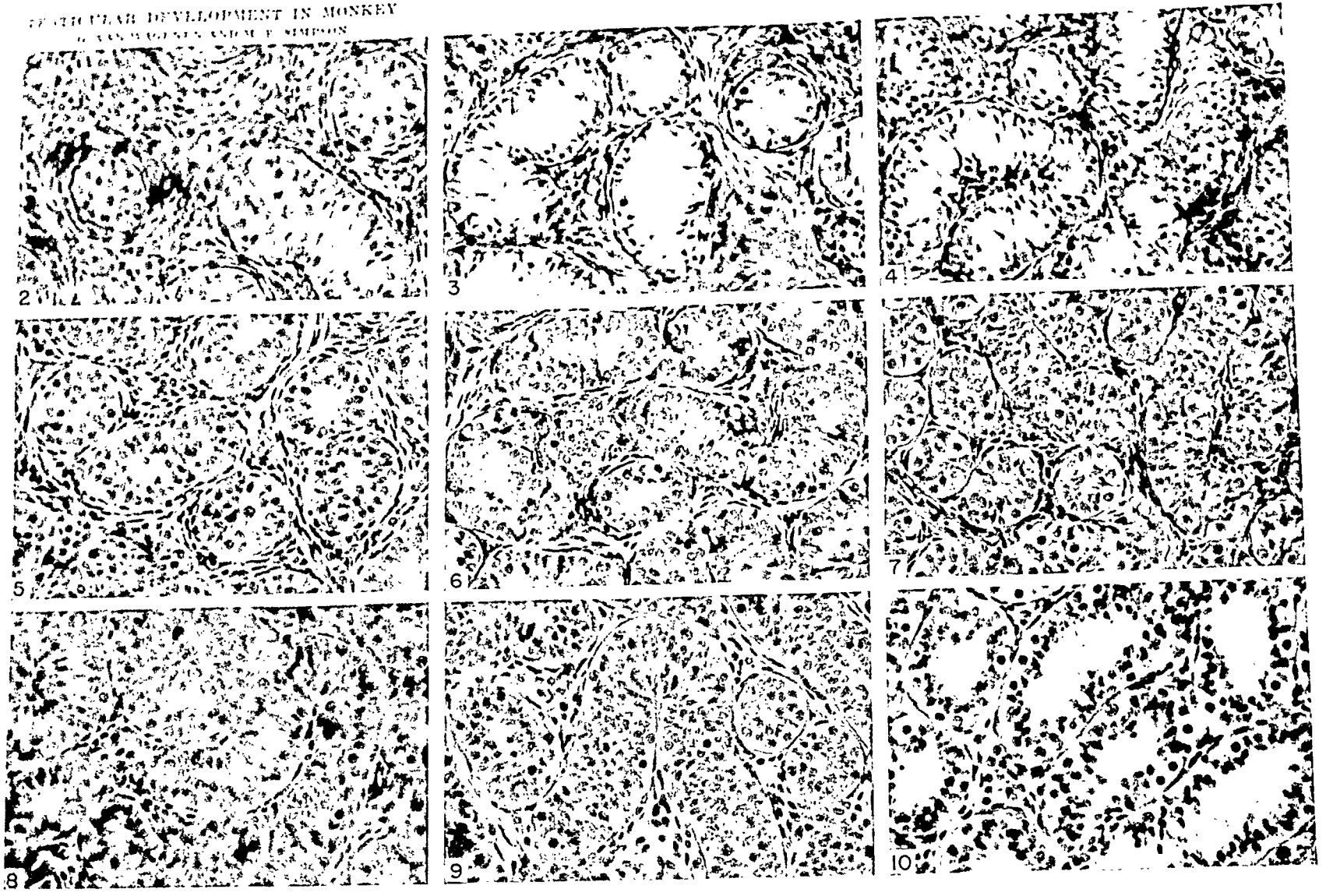

PLATE 2

EXPLANATION OF FIGURES

Figs. 11 to 14 Series of developmental stages in testis of an individual monkey (Mm *642*). × 87.

11 Right testis biopsy at 2 yr. 4 mo. 11 da. H and E. Tubules long, convoluted, closely packed 50–70 μ in diameter. Sertoli cells poorly differentiated, but cytoplasm is increasing and nuclei have partially moved basally. Interstitial cells small and sparse.

12 Right testis biopsy at 2 yr. 9 mo. 16 da. H and E. The changes are slight. Tubules are somewhat larger (70 μ) and Sertoli nuclei are more basal. No differentiating of Leydig cells seen.

13 Right testis biopsy at 3 yr. 1 mo. 26 da. H and E. Tubules have increased slightly in diameter (80–90 μ). Sertoli nuclei are now definitely basal. A few cells, recognizable as spermatocytes I by the spireme, are present. Interstitial cells still not clearly recognizable.

14 Left testis at 3 yr. 2 mo. 14 da. Mallory stain. Tubules measure 100–120 μ. Many spermatocytes are now present. Canalization is seen in a few tubules. A few epithelioid Leydig cells, singly or in pairs, are present.

Figs. 15 to 18 Series of developmental stages in macaque testes at higher power, × 237.

15 Testis biopsy (Mm *282*) at 2 yr. 7 mo. 14 da. H and E. Tubules measure 70 μ. Sertoli nuclei are basal. There are a few desquamating cells in the meshes of Sertoli cytoplasm. Occasional Leydig cells are recognizable.

16 Testis biopsy (Mm *282*) at 3 yr. 0 mo. 17 da. H and E. Tubules measure 70–80 μ. Sertoli nuclei are basal. Vascularity has increased, and there is more space between tubules. Epithelioid Leydig cells are present though not of mature size. Spermatocytes are appearing.

17 Left testis (Mm *642*) at 3 yr. 2 mo. 14 da. (See fig. 13.) Mallory stain. Tubules measure 100–120 μ. Spermatocyte I formation is here abundant.

18 Testis biopsy (Mm *282*) at 3 yr. 5 mo. 28 da. H and E. Tubules measure 130–150 μ. Many spermatids and some sperm are present. Some desquamation of immature cells is still present in the tubular lumen.

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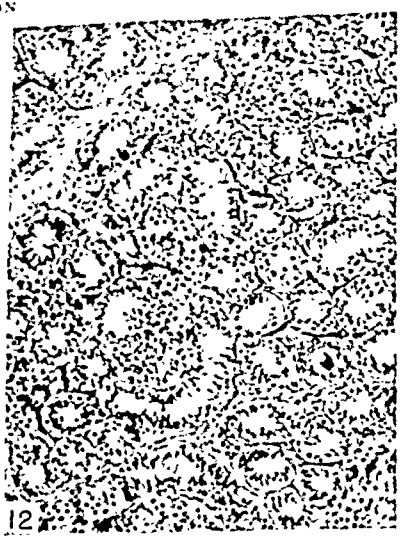

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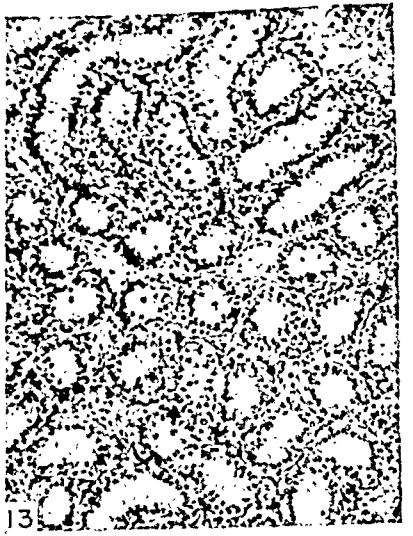

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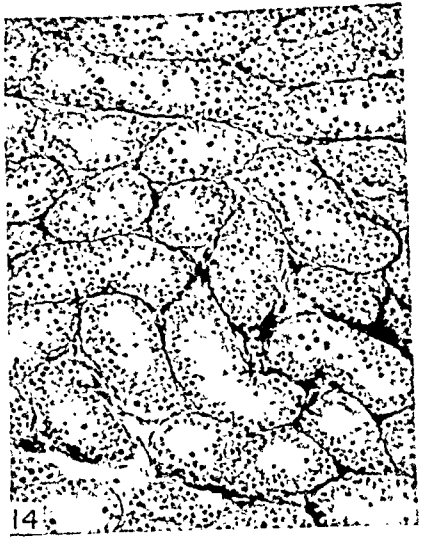

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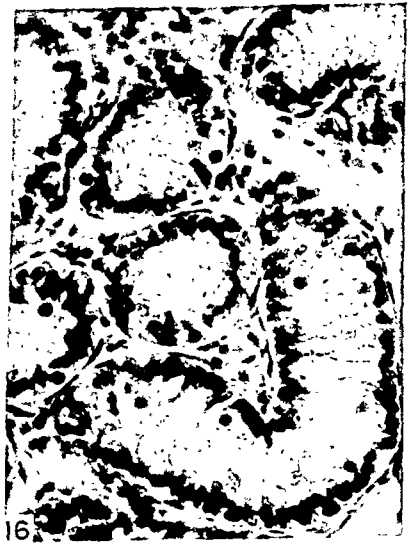

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PLATE 3

EXPLANATION OF FIGURES

19 Testis Mm *497*, 110 da. fetus, H and E × 87. Shows short, uncoiled tubules, diameter 70–80 μ. Sertoli cell cytoplasm is well developed and largely fills lumen. The broad intertubular spaces contain abundant cells, many of which are enlarged and rounded.

20 Testis Mm *497*, 110 da. fetus, H and E × 237. Shows tubules in cross section. The orientation of the interstitial tissue concentrically around the tubules, also the enlargement of the cells within the concentric rings, is shown.

21 Testis Mm *601*, birth, 174 days' gestation, H and E × 87. Shows Sertoli cell nuclei remaining in a basal position and a persistent wide intertubular space; however, Leydig cell size has decreased.

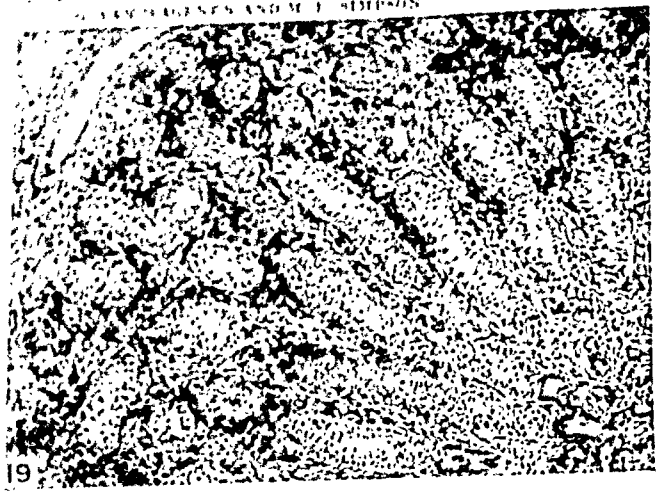
Figs. 22–25 Late developmental stages in testis of Mm *686*. Iron Haem. × 87.

22 Right testis sample at 2 yr. 10 mo. 3 da. Tubule diameter 100–120 μ. Spermatids have differentiated to presperm or almost mature sperm. Great amounts of cellular debris fill lumen. Few Leydig cells are differentiated.

23 Right testis sample one month later, at 2 yr. 11 mo. 1 da. Tubule diameter 100–140 μ. Sperm are present.

24 Right testis sample after another month, at 3 yr. 0 mo. 1 da. Tubule diameter 120–150 μ. Tubules are tightly packed and it is difficult to find Leydig cells. Cellular debris has been cleared from most tubules and a new generation of spermatids with orderly arrangement is present.

25 Right testis sample five months later, at 3 yr. 5 mo. 1 da. Tubule diameter 150 μ. Leydig cells are very rare. There are many presperm, and a few mature sperm free in the lumen.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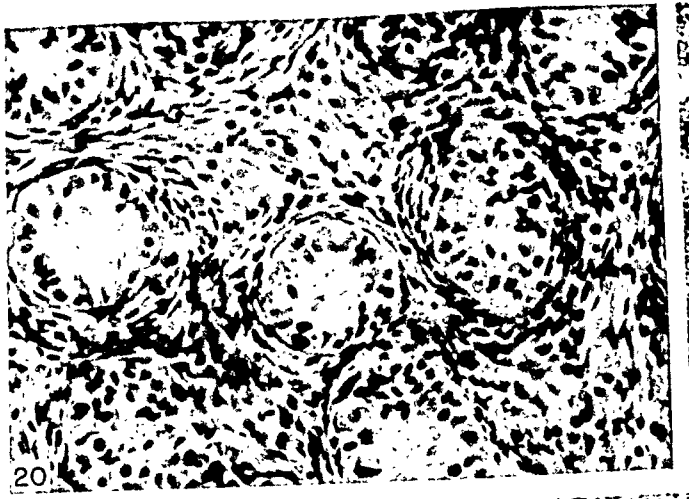
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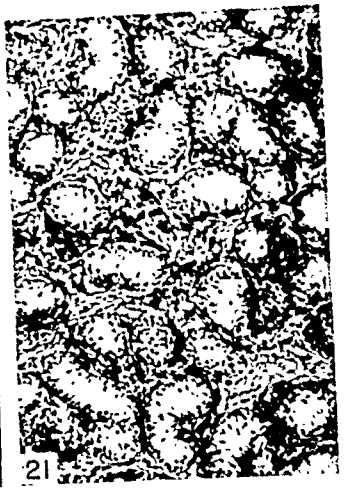
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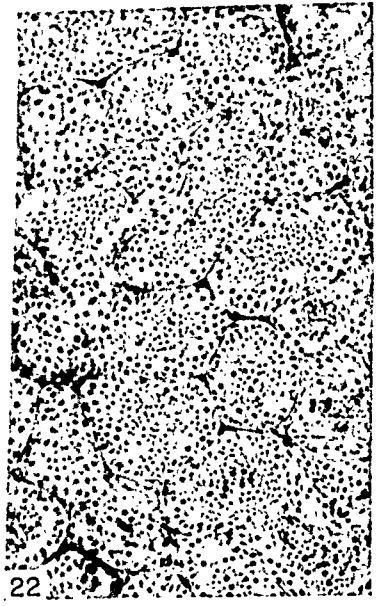
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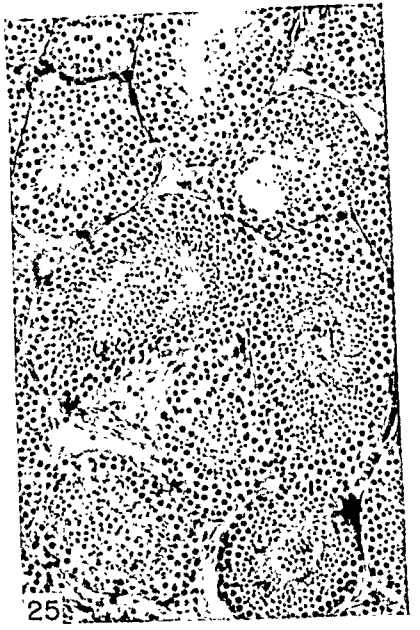
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PLATE 4

EXPLANATION OF FIGURES

Figs. 26 to 28 Adult testis, Mm *590*, age 11 yr. 6 mo. 20 da., H and E × 237.

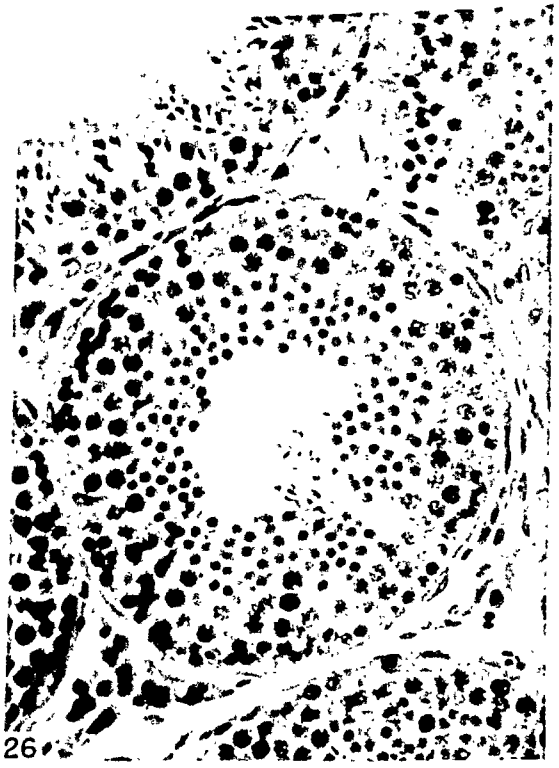
26 Seminiferous tubule lumen lined by young spermatids.

27 Tubule lined by spermatids with sperm-like heads.

28 Tubule containing mature sperm about ready to be shed.

TESTICULAR DEVELOPMENT IN MONKEY

H. VAN WAGENEN AND M. E. SIMPSON



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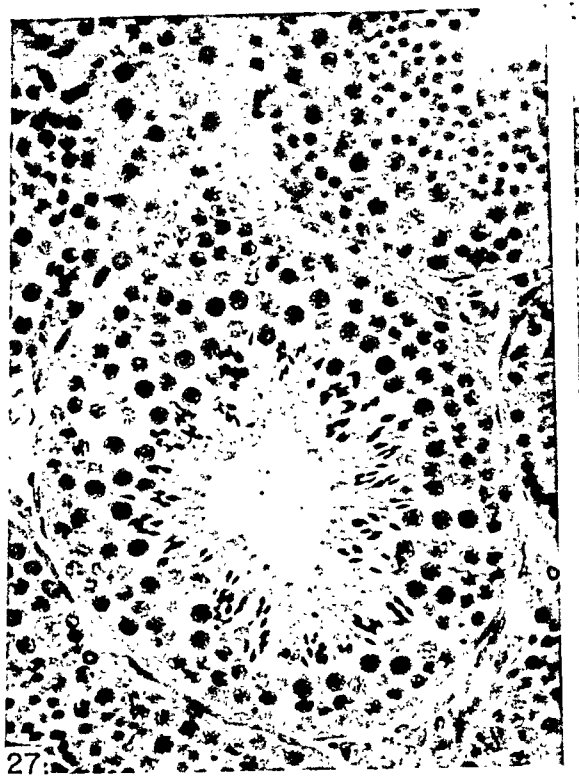

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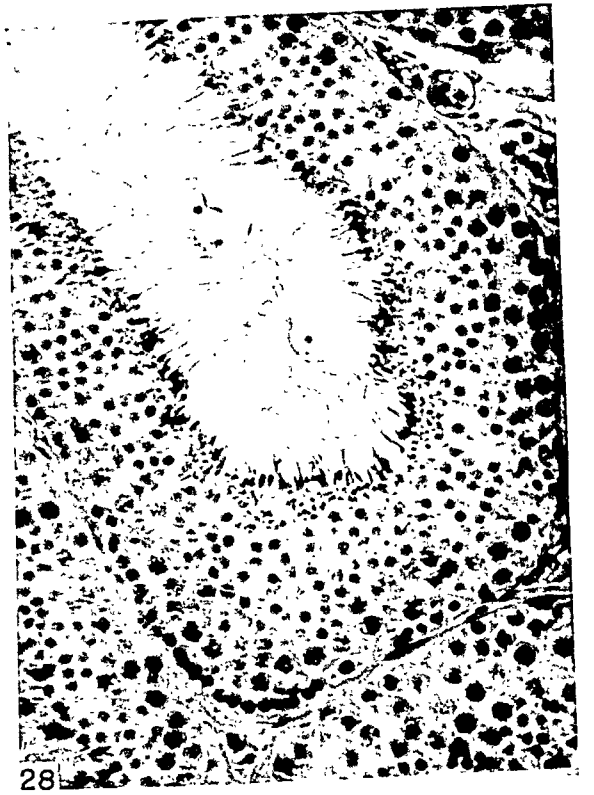

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AN AUTORADIOGRAPHIC STUDY OF THE DISTRIBUTION OF POLONIUM IN THE RAT DURING 24 HOURS AFTER INTRAVENOUS INJECTION [1]

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TWENTY-THREE FIGURES

Several natural radioactive elements emitting alpha particles have been shown to contain aggregates in the liquid state (reviewed by Schweitzer and Jackson, '52), and to deposit in the reticulo-endothelial tissues of various animals (Endicott and Yagoda, '47). For example, Arnold ('51) has demonstrated the deposition of uranium in aggregate form in tissues, and similar phenomena have been reported for radium (Schaefer, '47). The fact that polonium forms aggregates of considerable size *in vitro* (several hundred to several thousand atoms) is now well established (Chamie and Haissinsky, '34; Boyd, '49). In addition, a considerable amount of biological work has been done with this element

[1] This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester School of Medicine and Dentistry, Rochester, New York, and has appeared as a University of Rochester Atomic Energy Project Report, UR-220.

[2] This work was done in part during the tenure of an AEC Radiological Physics Fellowship and submitted in partial fulfillment of the requirements for the degree Master of Science, Department of Radiation Biology, University of Rochester, June, 1952.

[3] The cooperation of Dr. Marshall Brucer, Director, Medical Division, Oak Ridge Institute of Nuclear Studies, in making possible the completion of this work in his laboratory is greatly appreciated.

(Fink, '50; Lacassagne, '35; Finkel, Kisieleski, Hirsch and Mulhaney, '49) both for its intrinsic interest as an exceedingly toxic element and the facts that it has a convenient half-life and decays by almost pure alpha emission (giving off less than 10 quanta of gamma rays per million alpha particles) to a stable isotope of lead without intermediate radioactive decay products to complicate an experimental problem. Polonium is a useful isotope for autoradiographic studies because of the ease of identification of the alpha tracks, high resolution, and low background of random grains.

The presence or absence and the relative quantity of such aggregates could be expected to play an important role in the biological action of polonium. Firstly, such aggregates, when present, would be phagocytized rapidly and probably lead to considerable radiation doses to elements of the reticuloendothelial system. Secondly, the overall distribution and metabolism of the element and its ultimate toxicity might be dependent upon and altered considerably by the proportion of aggregated to non-aggregated atoms present. The experiments reported here were designed to study in some detail the behavior of polonium in the rat with respect to its distribution and aggregate formation during the first 24 hours after intravenous injection.

METHODS

Twenty-eight young adult albino rats of the Wistar strain were used in this study. The amount of polonium present in the tissues was measured radio-chemically and an autoradiographic study was made of samples from the same tissues after sectioning and preparation for microscopic examination.

Approximately 100 microcuries of polonium chloride per kilogram of body weight were injected into the caudal vein. Details of the injections were similar to those described by Fink ('50) and Casarett ('52) except that the stock solutions for injection were prepared in 0.5 N HCl and were injected without neutralization. Correlative studies indicate that neu-

tralization may increase the number of aggregates present in the injection solution. The volume of injection was approximately 1 ml per kilogram. This amount of 0.5 N HCl showed no clinical effect on the animals. Clean but not sterile techniques were employed.

The animals were sacrificed in pairs (by a blow on the head) at 2, 4, 6, 8, 10, 20, 40, and 60 minutes and at 2, 4, 6, 10, 15, and 24 hours after injection. Blood samples were drawn by cardiac puncture immediately after sacrifice using a heparinized syringe. Blood, liver, spleen, kidney, and muscle were taken at each sacrifice time for autoradiographic and radio-chemical analysis.

The tissue samples to be analyzed radio-chemically [4] were digested in concentrated nitric acid following the general techniques described by Fink ('50) except that perchloric acid was omitted. This modified procedure has been described by Scott and Stannard ('53) and appears to yield comparable polonium recoveries from rat tissues. After digestion the polonium was deposited on silver foils as described by Fink ('50, pp. 18–27); the alpha activity determined by conventional methods in a parallel plate ionization chamber. The alpha count was predetermined at 6400 and the background averaged two counts per minute leading under our experimental conditions to a standard deviation of 1.2%. The errors in digestion and other steps are considerably greater than this and should be considered to be at least $\pm 10\%$ (standard deviation) of the polonium concentration injected. This latter figure was obtained by analysis of "dummy injections" in which the same quantity of polonium as injected into the animal was injected into a bottle containing 100 ml of 0.5 N HCl. Details of these procedures are described elsewhere (Gallimore, '52).

For the autoradiographic study, a small piece of each tissue was fixed in 10% formalin, dehydrated, imbedded in

[4] The assistance of Mr. DeWitt Wood in the tissue digestions and of Mrs. Helen Haydon in the alpha counting, both in the Radiation Toxicology Section, University of Rochester Atomic Energy Project, is greatly appreciated.

paraffin and the autoradiograms made by the technique of Endicott and Yagoda ('47). For preparation of the blood autoradiograms, a small drop, approximately 0.005 ml, was placed on an 18 × 18 mm cover glass, pulled in the manner conventionally used for preparing blood smears and then both cover glasses placed in apposition to the nuclear emulsion. NTA emulsion was used throughout and all exposures carried out in light-tight boxes placed in a refrigerator at about 5°C. The tissue autoradiograms were exposed for 15 days, the blood smears for 16 days.

A possible source of error in the autoradiographic study may occur in preparation of the tissues. Since conventional histological processing was carried out there is the possibility of leaching of the radioactive material into the processing solutions. However, we believe that this does not constitute an important factor in this study since there was relatively little "smearing" of the images and very little activity was found in the processing solutions. This is supported also by work on gold colloid autoradiograms in which the results of freeze drying and formalin fixation methods were compared (Stembridge, '52).

To insure ready comparison of the photomicrographs made from the autoradiographs, the field to be photographed was projected and focused on a ground glass plate placed at the film level. A spot was chosen containing tissue devoid of alpha tracks and the light intensity measured with a "Densicron" densitometer. Suitable photographic exposure time was then determined. Further details concerning these and related procedures can be found elsewhere (Gallimore, '52).

RESULTS

Both autoradiographic and radio-chemical data are presented in terms of changes as a function of time.

Distribution of polonium in tissues

The relative amount of polonium appearing in the tissues sampled is presented in terms of a modified differential

absorption ratio, DAR. The conventional DAR (Marinelli, Quimby and Hines, '48) defined as the ratio of the concentration of the isotope in the tissue to the average concentration in the body, could not be calculated here since the residual carcass of the animals was not digested and analyzed for polonium content. Instead, the quantity:

$$\frac{\text{activity in tissue sample/sample weight}}{\text{activity administered/body weight}}$$

was calculated. This is permissible in this case because polonium is eliminated from the body with an effective half-life of approximately 30 days (Hursh and Stannard, '48). Therefore during the first day the activity administered represents a reasonable approximation to the activity present in the body. This method of presentation serves to emphasize the tendency of an element to be concentrated in a given tissue and the changes in relative concentration with time.

The concentration of polonium in spleen, blood, liver, and kidney in each of the two rats sacrificed at the time intervals previously enumerated is represented in figures 1 and 2. The data are expressed as the modified DAR values described above.

While there are some inconsistencies, it appears that the relative proportion of polonium in blood reaches a peak during the first 10 minutes after administration and then falls gradually. The maximum DAR value may be considered to occur at the time of complete mixing of the administered polonium with the blood.[5] The changes in blood concentration *per se* are depicted in the disappearance curve plotted as figure 3.[6] Comparison with excretion measurements (Fink, '50, pp. 40–53) shows that the rate of disappearance of polonium from blood is very much more rapid than its excre-

[5] For example, Cartland and Koch ('28) in determining the blood volume of rats using the dye dilution method stated that two minutes was sufficiently long for complete mixing of the dye while Went and Drinker ('29) in similar studies estimated the mixing time as about 4½ minutes.

[6] The low point at 20 minutes may not be real since one rat showed quite low DAR values in blood (also kidney and spleen) at this time.

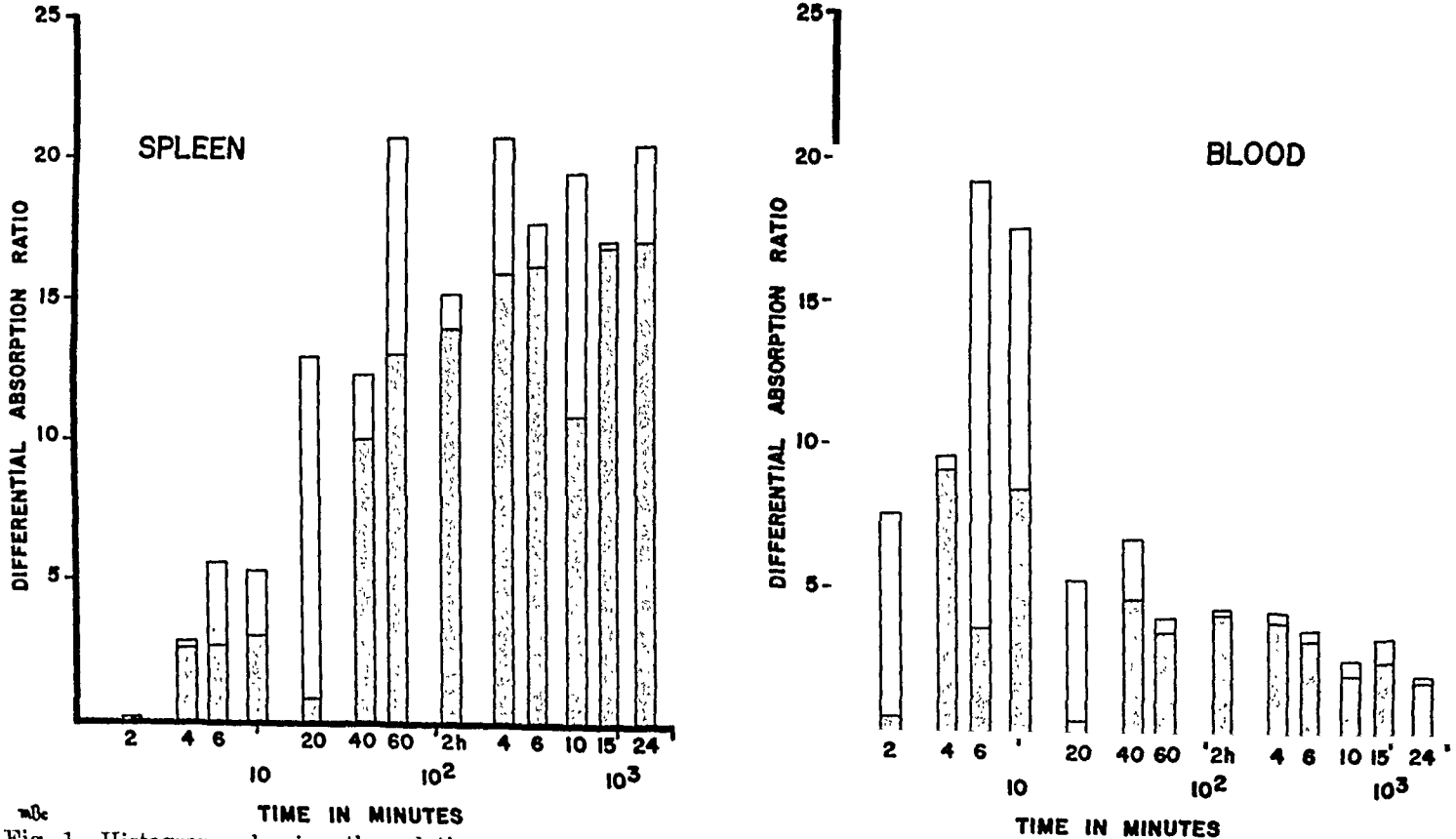
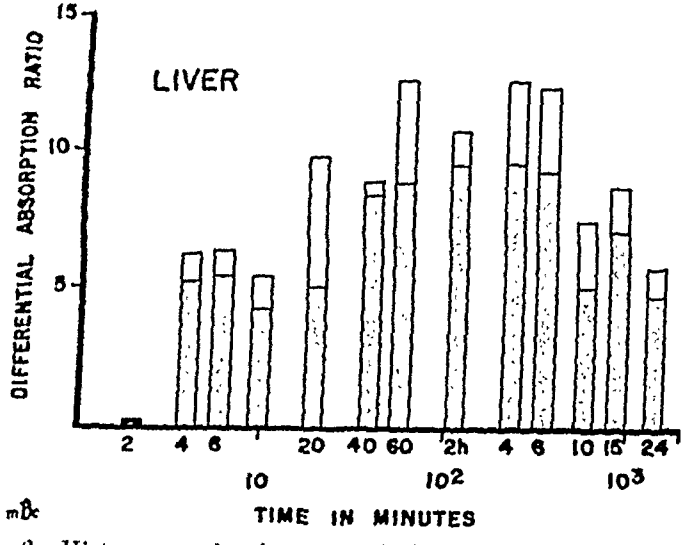


Fig. 1 Histograms showing the relative concentration of polonium in rat spleen and whole blood. The abscissa represents time in minutes and hours (indicated by h); the ordinate presents the differential absorption ratio (see text). The bars represent two rats, the clear area indicating the amount the higher animal exceeded the lower. The time scale emphasizes the changes occurring in the first hour.



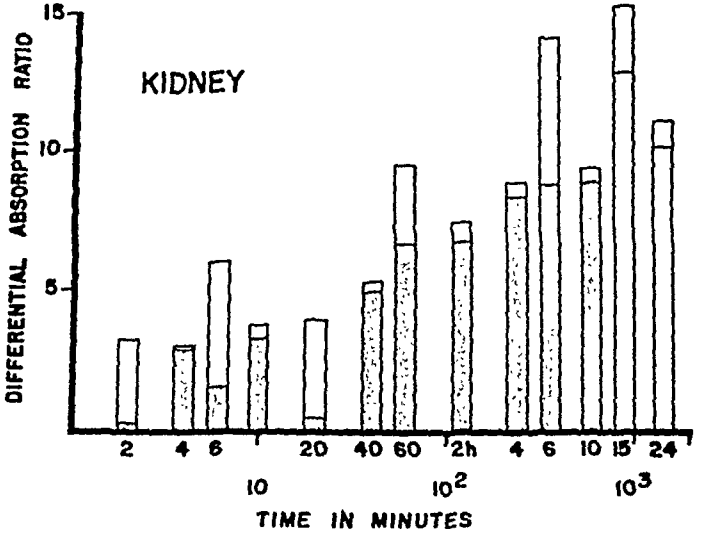


Fig. 2 Histograms showing the relative concentration of polonium in liver and kidney of the rat. See explanation of figure 1.

tion from the body. Thus migration into the tissues is to be expected.

Further examination of the data in figures 1 and 2 reveals several features of the migration of this element into the tissues. The spleen, liver, and kidney continue to gain in

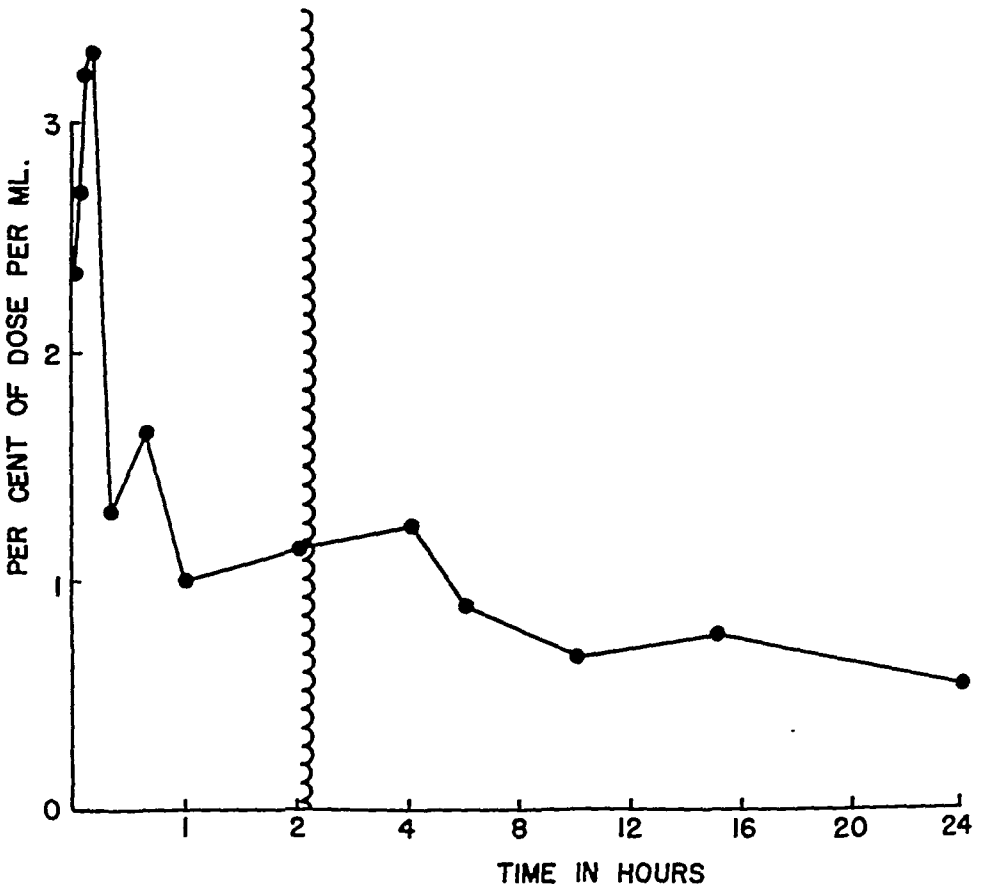


Fig. 3 The disappearance of polonium from rat blood during the first 24 hours after intravenous injection. Each point represents the average of two rats.

relative concentration of polonium for considerable periods after the blood content has begun to fall. Spleen retains its relatively high concentration or may even increase slightly to 24 hours. Kidney shows a definite tendency to concentrate polonium up to about 15 hours, at least, but in contrast to spleen and liver, the kidney DAR values rise relatively slowly.

Not shown in the figures is the fact that there is little accumulation of polonium in muscle after the first 10 minutes. The DAR values averaged 0.18 from 10 minutes to 24 hours with a range of individual observations from 0.08 to 0.27.

For reference purposes, the data from the present study are compared in table 1 with data obtained in earlier work. Both are expressed in terms of per cent of dose per gram wet weight found in the tissues 24 hours after intravenous administration. The agreement is considered within the error of the methods employed.

TABLE 1

Percentage of dose per gram (wet weight) found in rat tissues 24 hours after intravenous administration of polonium chloride

TISSUE	PRESENT DATA (Avg. of two rats)	FINK (1950) (Avg. of five rats)
Whole blood	0.5	1.1
Liver	1.4	1.7
Spleen	5.1	7.6
Kidney	2.8	2.8
Muscle	0.1	0.1

Autoradiographic data

Representative low power photomicrographs prepared as described above are shown in figures 4 through 23.[7] Attention is invited to two phenomena: (a) the presence of discrete black spots (sunbursts) which we interpret as indicating the presence of aggregates, probably of colloidal dimensions, and (b) a diffuse blackening which can be considered as tracks from randomly distributed atoms of polonium.

Figures 4–7 show representative autoradiograms of the blood. From two to 10 minutes after injection there are many sunbursts which vary widely in size and may actually be of two different types (Gallimore, '52). In addition, many

[7] The figures chosen represent typical appearances which were duplicated with acceptable consistency.

randomly distributed alpha tracks can be seen. Examination of the later autoradiograms seen in figures 5, 6, and 7 shows a gradual loss of the large sunbursts as a function of time. In fact, from 2–3 hours to termination of the experiment they are rarely seen and the blood smear autoradiograms present an appearance like that seen in figure 7. No quantitative estimates have been possible but it seems clear that the random single track density does not decrease as much as does the frequency of occurrence of sunbursts.

Examination of the tissue autoradiograms, figures 8–23, shows that the aggregates are progressively concentrating in the tissues, particularly those of the reticulo-endothelial system. Also the course of polonium excretion by the kidney can be followed.

1. The appearance of kidney tissues may be seen in figures 8–14. In the animals sacrificed at two minutes there are polonium atoms in the glomeruli and the vascular bundles penetrating into the medulla (figs. 8–9). At 4 minutes the element begins to appear in the proximal convoluted tubules but the greatest portion is still seen associated with the vascular structures (figs. 10–11). By 20 minutes the deposition in the proximal convoluted tubules has increased to approximate that in the glomerulus (fig. 12). In contrast, the amount associated with medullary blood vessels begins to decrease. This is quite evident by one hour (fig. 13). On the other hand, the tubules continue to concentrate the material until by 24 hours (fig. 14) most of that seen in kidney cortex is outside the glomerulus.

Not clearly shown in the figure but visible in the originals is the fact that the material in the tubules appears to be largely present in the form of single atoms, *not* as aggregates.

2. The deposition in spleen is hardly apparent at two minutes (fig. 15), but the presence of aggregates is definite in the 4-minute animals (fig. 16). By 40 minutes the larger spots show a ring-like pattern (fig. 17) and appear to have deposited at the marginal zone of the germinal centers. There is also a rather heavy deposition of randomly distributed atoms in the red pulp.

The tendency of the spots to locate as described above continues as seen in the autoradiograms at 10 and 24 hours (figs. 18–19). However, the black spots are less discrete and, under higher power, show evidence of disintegration.

3. The changes described above in spleen may be seen perhaps somewhat more clearly in liver (figs. 20–23). A few aggregates are seen at two minutes (fig. 20); they are highly localized and discrete at two hours (fig. 21), less definite at 10 hours (fig. 22), and diffuse by 24 hours (fig. 23). This process of disintegration of the aggregates appears to be a real phenomenon and its occurrence in this study confirms an earlier, smaller, unpublished series made by Boyd using high magnification.

4. Examination of the autoradiograms of muscle showed only an occasional aggregate associated with a blood vessel, and a preponderance of single tracks at all times.

DISCUSSION

There seems to be little doubt that polonium chloride administered intravenously exists in circulating blood of the rat in two systems: one, a system of aggregates characterized by sunbursts in the autoradiograms and the other a system of non-grouped singly dispersed atoms. The aggregates are removed from the blood stream in about two hours and deposited in the liver and spleen; behavior consistent with the activities of the reticulo-endothelial system. Both liver and spleen seem to be able to disperse the aggregates. This begins, in the case of liver, at about 10 hours after intravenous administration and somewhat later in the spleen. The total concentration relative to that in the rest of the body continues high in the spleen up to 24 hours whereas the relative amount in liver begins to decrease somewhat. Since the aggregates do not reappear in the blood or muscle, it is assumed that they are being destroyed. Evidence of their disintegration was seen microscopically. The fact that the concentration of random atoms in the proximal convoluted tubules of the kidney relative to those in the glomeruli increases continuously with time suggests that some of the polonium from the broken-up aggregates in the liver and spleen may have entered the blood stream and become deposited at this site.

The extent to which the aggregates seen in the autoradiograms were present in the original injected solution cannot

be stated. Some were probably present but there is also evidence pointing to the formation of additional aggregates when polonium solutions are added *in vitro* to rat plasma (Boyd and Williams, unpublished). At the pH of the injection solution the polonium probably existed primarily as a cation, but at neutrality it may be partly or largely anionic (Haissinsky, '36). Further work will be necessary to elucidate these points and to find whether or not there are differences, either physical or physiological, between aggregates from the two sources.

It may be noted that many more aggregates are present in the blood and tissues of rats receiving polonium intravenously than when the material is administered orally. For example, Boyd, Stannard and Williams ('48) could not find polonium aggregates in the blood of rats fed fairly large doses of polonium by gavage. The observations covered the period from 7 minutes to 24 hours. Aggregates were found in the feces but only single tracks in the blood smears. Of further interest in this regard is the fact that the distribution of polonium in the body is found to be rather different after oral than after intravenous administration (Fink, '50; and Stannard, '49, pp. 55–57). Particularly the relative amounts in kidney, spleen, and liver during the first days are much lower after oral than after intravenous administration. While other differences such as the maximum concentrations reached, etc., may exist between these two routes of administration, the relative numbers of aggregates present would be expected to contribute variations in toxicity and pathology. Such data have been gathered in other laboratories but are not available for publication at the present time.

Bloom ('48, p. 543) commented upon the fact that most of the radioactive materials studied in the Plutonium Project at the University of Chicago were distributed diffusely and homogeneously in the liver. Plutonium and radium, however, exhibited marked tendencies to form clumps. For example, in autoradiographs of livers from mice injected intravenously with plutonium, the pattern changed gradually from a

relatively diffuse one at 6 hours to the presence of large agglomerated masses at 42 days. In the present study, polonium resembles plutonium and radium in its tendency to form aggregates. However, these disappear from the liver much more rapidly than aggregates of plutonium or radium. In our studies polonium seems to resemble plutonium and radium (Bloom, '48, p. 283) in its gross behavior in the spleen.

Since autoradiography is not a quantitative method, present data do not allow a strict accounting of the various transitions which polonium undergoes in the body of the rat as a function of time. However, the findings can be considered as qualitatively confirming the postulate that there are fairly large differences in the manner in which aggregated material and single atoms are metabolized during the first day after administration. The need for careful analysis and control of the properties of injection solutions is clear. Obviously, this applies not only to polonium, but to other radioactive materials. Indeed it may apply as well to non-radioactive elements in solution.

SUMMARY

The distribution of polonium chloride in the liver, spleen, kidney, muscle, and blood of rats after intravenous administration has been studied autoradiographically and radiochemically.

It was found that the polonium in the blood, immediately after injection, existed in two systems; a system of polonium aggregates, characterized by sunbursts in the autoradiograms and the other a system of non-grouped singly dispersed atoms or molecules.

The aggregates were removed from the blood stream over an approximate two-hour period and deposited in the liver and spleen.

The non-particulate polonium was observed to concentrate in the proximal convoluted tubules of the kidney. No particulate polonium was observed to be deposited in this organ.

The polyatomic groupings deposited in liver and spleen are seen to become diffuse and to gradually disintegrate beginning at about 10 hours after administration and continuing to the end of the observation period (24 hours).

It is concluded that the aggregates are taken up by elements of the reticulo-endothelial system and inferred that distribution, excretion, and toxicity may be affected by the relative number of aggregated and dispersed atoms present under any given conditions.

LITERATURE CITED

ARNOLD, JAMES S. 1951 Exhibit-radioautographs of U 233 in bone and soft tissue. Atomic Energy Commission declassified report AECD-3176 *. Microcard issued March, 1951, declassified June 6, 1951.

BLOOM, WILLIAM 1948 Histopathology of Irradiation from External and Internal Sources, ed. by William Bloom, National Nuclear Energy Series, vol. IV-221, McGraw-Hill Book Co., New York, 808 pages.

BOYD, G. A. 1949 Autoradiographic techniques. Nucleonics, *4:* No. 1, 40.

BOYD, G. A., J. N. STANNARD AND A. WILLIAMS *1948 Aggregates of polonium* in blood and their possible biological significance. Paper given at Atomic Energy Commission Information Meeting, Chicago, Illinois, October 18, 19, and 20, 1948.

CARTLAND, G. F., AND F. C. KOCH 1928 A micro-modification of the Keith-Rowntree plasma-dye method for the estimation of blood volume in the rat. Am. J. Physiol., *85:* 540–545.

CASARETT, G. W. 1952 Histopathology of alpha radiation from internally administered polonium. University of Rochester Atomic Energy Project, Report UR-201,* in two parts, issued August 25, 1952, 563 pages.

CHAMIE, C., AND M. HAISSINSKY 1934 Effects of age and polonium content on centrifugation of polonium solutions. Inst. de France, Compt. Rend., *198:* 1229–1231.

ENDICOTT, K. M., AND H. YAGODA 1947 Microscopic historadiographic technic for locating and quantitating radioactive elements in tissues. Proc. Soc. Exp. Biol. Med., *64:* 170–172.

FINK, R. M. 1950 Biological Studies with Polonium, Radium, and Plutonium, ed. by R. M. Fink. National Nuclear Energy Series, vol. VI-3, McGraw-Hill Book Co., New York, 411 pages.

FINKEL, M. P., W. E. KISIELESKI, G. M. HIRSCH AND T. J. MULHANEY 1949 Polonium and CF-1 female mice: The 30-day LD_{∞}, retention, distribution, and concentration. Argonne National Laboratory, Report ANL-4333,* 84–96.

———— 1952 Am. J. Roent., Radium Therapy, and Nuclear Med., *LXX:* 477–485.

GALLIMORE, J. C. 1952 A radiochemical and autoradiographic study of the distribution of polonium in rats after intravenous administration. University of Rochester Atomic Energy Project, Report UR-220,* 39 pages. Also Thesis, Department of Radiation Biology, University of Rochester.

HAISSINSKY, M. 1936 Electrochemistry of Polonium. Trans. Electrochem. Soc., *70:* 29.

HURSH, J. B., AND J. N. STANNARD 1948 Present status of polonium tolerance estimations. University of Rochester Atomic Energy Project, Report UR-44,* issued October 14, 1948, and declassified in 1953, 33 pages.

LACASSAGNE, A., AND M. SERVIGNE 1935 Localization of polonium in the organism dependent on the nature of the solution injected. J. de Pharmacie et de Chimie, *21:* 145–151.

MARINELLI, L. I., E. H. QUIMBY AND G. J. HINES 1948 Dosage determination with radioactive isotopes. II. Practical considerations in therapy and protection. Am. J. Roent. and Radium Therapy, *59:* 260.

SCHAEFER, HERMANN 1947 Nachweis und Messung kleinster Alpha-Aktivitäten in biologischen Substanzen durch Bahnspuraszählung in der Photoemulsion. Strahlentherapie, *77:* 613–628.

SCHWEITZER, G. K., AND M. JACKSON 1952 Radiocolloids. J. Chem. Educ., *29:* 513–522.

SCOTT, R. G., AND J. N. STANNARD 1953 Modified procedure for analysis of polonium ^{210} in biological materials. University of Rochester Atomic Energy Project, Report UR-235,* 22 pages.

STANNARD, J. N. 1949 In: Summary of research and service programs. University of Rochester Atomic Energy Project, Report UR-60,* issued March 28, 1949, 55–57.

STEMBRIDGE, V. 1952 Unpublished observations at Medical Division, Oak Ridge Institute of Nuclear Studies.

WENT, S., AND C. K. DRINKER 1929 A micromethod for determination of the absolute blood volume, with data upon the blood volume of the guinea pig, white rat, rabbit and cat. Am. J. Physiol., *88:* 468–478.

* Obtainable from Technical Information Service, U.S.A.E.C., P.O. Box 62, Oak Ridge, Tennessee.

PLATE 1

EXPLANATION OF FIGURES

4 Typical autoradiogram of rat blood smear two to 10 minutes after intravenous administration of polonium chloride.

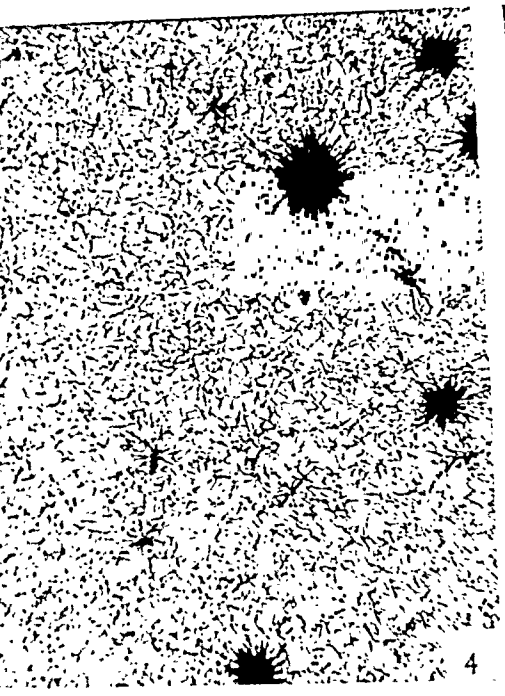
5 Typical autoradiogram of rat blood smear 10 to 40 minutes after intravenous administration of polonium chloride.

6 Typical autoradiogram of rat blood smear 40 minutes to two hours after intravenous administration of polonium chloride.

7 Typical autoradiogram of rat blood smear two hours to 24 hours after intravenous administration of polonium chloride.

NIUM AUTORADIOGRAPHY

LLIMORE, BOYD AND STANNARD



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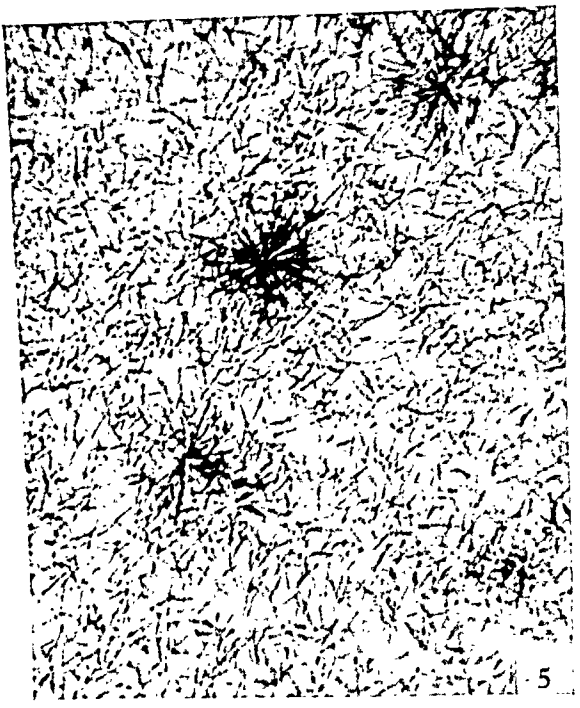

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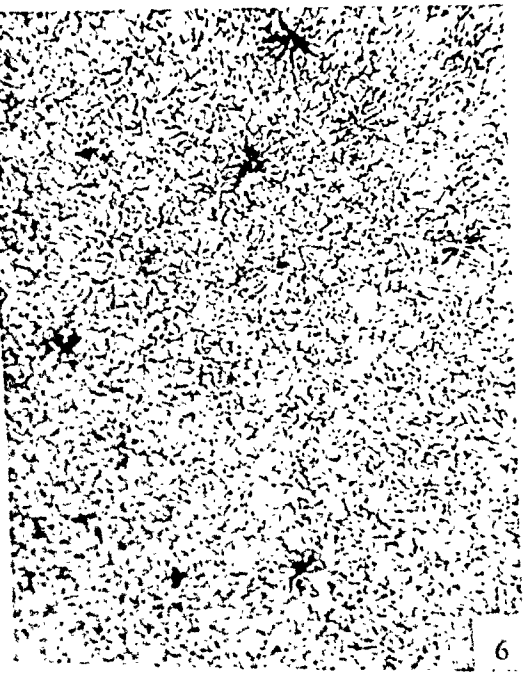

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PLATE 2

EXPLANATION OF FIGURES

8 Autoradiogram of kidney cortex at two minutes after intravenous administration of polonium chloride. × 120.

9 Autoradiogram of kidney medulla at two minutes after intravenous administration of polonium chloride. × 60.

10 Autoradiogram of kidney cortex at 4 minutes after intravenous administration of polonium chloride. × 120.

11 Autoradiogram of kidney medulla at 4 minutes after intravenous administration of polonium chloride. × 60.

12 Autoradiogram of kidney cortex at 20 minutes after intravenous administration of polonium chloride. × 120.

13 Autoradiogram of kidney medulla at one hour after intravenous administration of polonium chloride. × 60.

POLONIUM AUTORADIOGRAPHY
GALLIMORE, BOYD AND STANNARD


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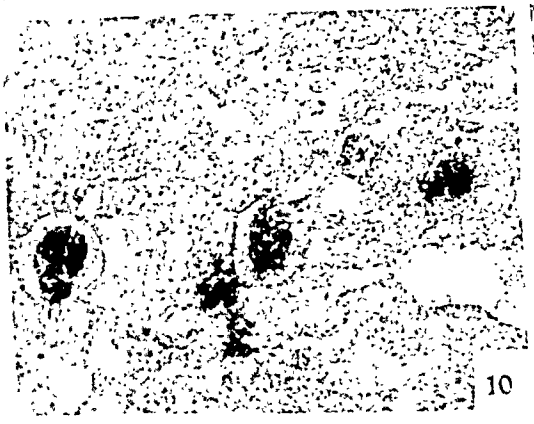
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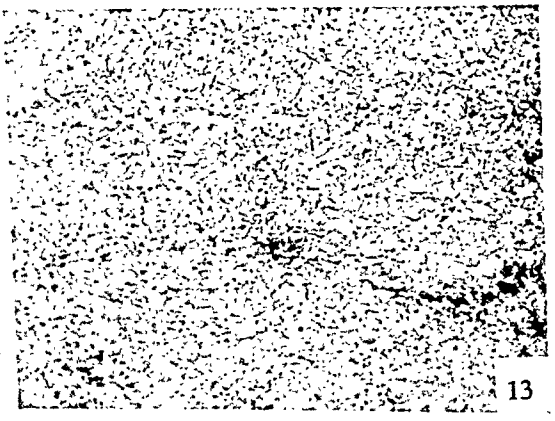
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PLATE 3

EXPLANATION OF FIGURES

14 Autoradiogram of kidney cortex at 24 hours after intravenous administration of polonium chloride. × 120.

15 Autoradiogram of spleen at two minutes after intravenous administration of polonium chloride. × 80.

16 Autoradiogram of spleen at 4 minutes after intravenous administration of polonium chloride. × 80.

17 Autoradiogram of spleen at 40 minutes after intravenous administration of polonium chloride. × 80.

18 Autoradiogram of spleen at 10 hours after intravenous administration of polonium chloride. × 80.

19 Autoradiogram of spleen at 24 hours after intravenous administration of polonium chloride. × 80.

POLONIUM AUTORADIOGRAPHY

GALLIMORE, BOYD AND STANNARD


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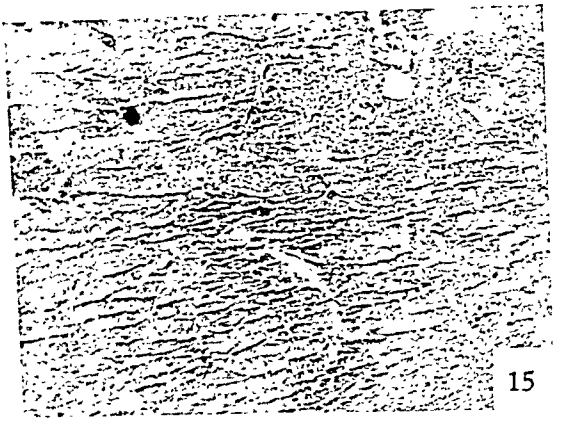
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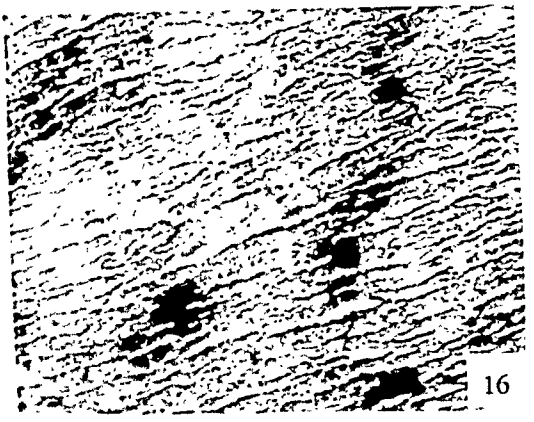
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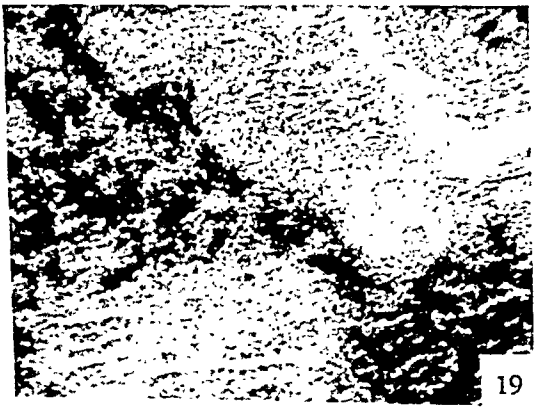
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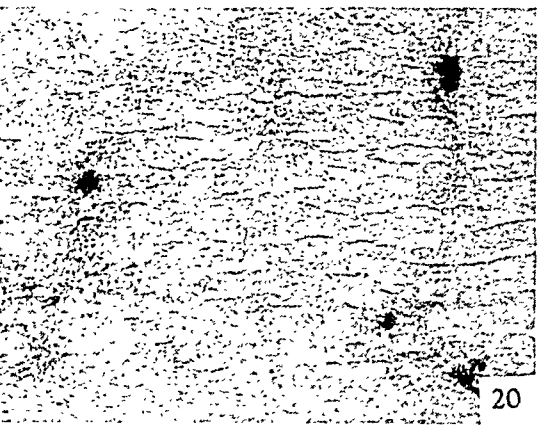

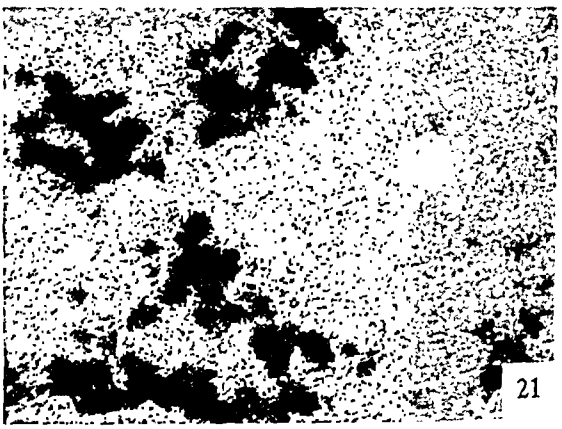

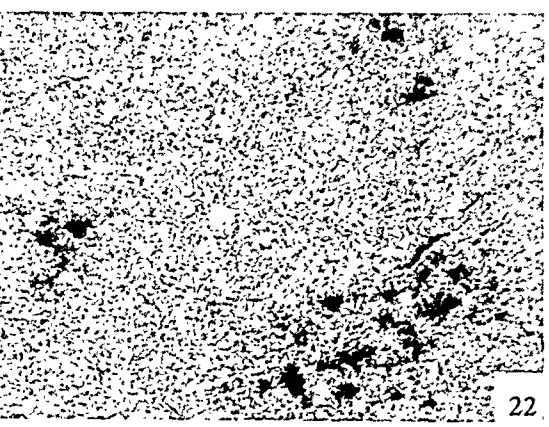

0 Autoradiogram of liver at two minutes after intravenous administration of polonium chloride. × 75.

1 Autoradiogram of liver at two hours after intravenous administration of polonium chloride. × 75.

2 Autoradiogram of liver at 10 hours after intravenous administration of polonium chloride. × 75.

3 Autoradiogram of liver at 24 hours after intravenous administration of polonium chloride. × 75.

AMERICAN ASSOCIATION OF ANATOMISTS

Sixty-seventh Annual Session

University of Texas
Galveston, Texas

April 7, 8, 9, 1954

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ABSTRACTS

Abstracts of papers presented at the 67th meeting of the American Association of Anatomists at University of Texas, Galveston, Texas, April 7, 8, 9, 1954.

The abstracts are listed in alphabetical sequence by senior author in 5 lists as follows: papers presented from platform, papers read by title, demonstrations, motion picture demonstrations, and Tissue Culture Association abstracts.

The numbers at the beginning of the abstracts refer to the order of presentation at the meeting, as printed in the program.

PAPERS FROM PLATFORM

55. *The nonessentiality of the hypophysis for the induction of tumors with 3–4 benzpyrene.*[1] Frederic J. AGATE, Jr., William ANTOPOL*, Susi GLAUBACH* and Fay AGATE, Department of Anatomy, Columbia University; Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital; and Department of Biochemistry, Francis Delafield Hospital.

Twenty hypophysectomized rats of the Long-Evans strain, 2 to 4 weeks after operation, were given a single subcutaneous injection of 10 mg of 3–4 benzpyrene in 1 cc of sesame oil. Twelve intact animals were similarly injected. The first definite tumor was palpable in the controls at 99 days after injection and the first palpable tumor was found in the hypophysectomized animals at 106 days. Seven hypophysectomized animals without tumors died before 93 days and before any of the intact rats developed tumors. One hypophysectomized rat died without a tumor on the 117th day post injection. The remaining twelve hypophysectomized animals all developed tumors as did all intact animals.

The tumor-bearing hypophysectomized rats died between the 180th and 231st day after injection with the mean falling at 194 days. The mean weight of the tumors was 28.2 gms. All but 3 of the intact rats survived until the 204th day when they were sacrificed. The mean tumor weight was 107.9 gms.

It is concluded that the hypophysis plays no determining role in the induction of neoplasm with 3–4 benzpyrene. It is evident that the growth of these induced tumors is somewhat slower in hypophysectomized than in intact rats.

[1] Supported in part by a grant from the American Cancer Society.

149. *Further observations on the biological activity of estradiol-sensitized collodion particles.* Roland H. ALDEN, Anna Dean DULANEY* and Mary GIDEON, Division of Anatomy and Division of Pathology and Bacteriology, University of Tennessee.

Crystalline alpha-estradiol[1] was dissolved in an acetone solution of collodion and the steroid incorporated in or onto the collodion particles in a manner previously reported (Proc. Soc. Exp. Biol. and Med., *74*, 466).

Evidence will be presented indicating that these estrogen-sensitized collodion particles (1) provide an efficient means of giving prolonged, steady hormone administration with single or few injections, (2) may be injected intramuscularly or subcutaneously with equally good results, (3) are reasonably stable for periods up to at least thirty months, (4) are probably stored in R-E cells near the site of injection from whence the hormone is slowly eluted.

[1] Schering Corporation kindly supplied the estrogen.

154. *Observations on the source of the sympathetic nerves to the ductus arteriosus in the human full term fetus.* Frank D. ALLAN*, Department of Anatomy, Louisiana State University School of Medicine. (Introduced by Charles M. Goss.)

Numerous investigators have demonstrated microscopically the presence of nerves in the wall of the ductus arteriosus. There is, nevertheless, a paucity of

information, especially in man, regarding the source of these nerves. In animals, fibers whose origin was determined to be the aortic (depressor) nerve of the vagus (Boyd, '41) have been described as terminating in the ductus. Superficial mention has been made of fibers, presumably sympathetic, passing to the ductus from the lower cervical trunk. Nonetheless, no systematic gross anatomical study has been made regarding these nerves.

Dissection of the nerves to the ductus in full term human fetuses demonstrated occasional branches reaching that structure from the left superior and inferior cervical cardiac sympathetic nerves. The usual source for such nerves, however, was the inferior cervical cardiac sympathetic nerves(s) on the left. Other sources were the left upper three thoracic cardiac sympathetic nerves.

Characteristically, the inferior cervical cardiac sympathetic nerve forms, on the proximal portion of the left subclavian artery, a plexus which yields filaments to the ductus; these are joined by contributions from the thoracic cardiac sympathetic nerves or additional fibers from the superior cardiac plexus. The plexus thus formed on the anterior and lateral aspects of the ductus extends to the pulmonary trunk, diminishing as it proceeds, and terminates by communicating with nerves associated with the left coronary artery.

115. Anatomical observations upon the vascular bed in the myocardium of the dog.[1] A. W. ANGULO, Division of Anatomy, Hahnemann Medical College.

The purpose of this presentation is to show the arrangement and distribution of arteries, veins and the capillary bed within the myocardium. The interrelationship of these vessels with each other and with the Thebesian veins will be discussed following the application of new methods.

[1] This investigation was supported in part by a research grant H-1366 from the National Heart Institute.

81. The cryptorchid testis in the development and maintenance of maleness as tested by the sexual behavior of the male guinea pig.[1] Harold R. ANTLIFF* and William C. YOUNG, Department of Anatomy, University of Kansas.

Studies of experimental cryptorchidism have been confined to effects on spermatogenesis, the reproductive tract and accessories in adults rather than to development from birth. Behavior has not been studied. It is believed by some that the abdominal placement of testes will not produce the endocrine disturbances associated with natural cryptorchidism, for the failure of descent is taken as an indication of congenital abnormality. Experiments designed at clarification have been undertaken.

Ten males were made bilaterally cryptorchird within 3 days after birth. Five intact and 3 castrated animals are controls. A first series of tests of sexual behavior was begun after weaning and isolation the seventeenth day and continued at weekly intervals until day 120. In an investigation of long time effects a series of 5 weekly tests was begun at 300 days of age.

The schedule of development of sexual behavior in the cryptorchids was not significantly different from the controls; average age at the first ejaculation by the cryptorchids was 65 days, that of intact controls 60 days. Average scores for

the 5 to 10 tests from the first ejaculation was 9.4 for cryptorchids and controls. Clearly, experimental cryptorchidism as such was without detectable effect on development and display of sexual behavior up to day 120. Results from tests begun day 300 will be reported.

[1] Aided by grants from the U. S. Public Health Service.

143. *Electron microscopic studies of experimentally induced changes in ground substance of loose connective tissue.* A. M. ASADI*, T. F. DOUGHERTY and George W. COCHRAN*, Departments of Anatomy, University of Utah College of Medicine, and Plant Pathology, Utah State College.

A new technique was developed for preparing samples of intact loose connective tissue for electron microscopy. Cuticles of loose connective tissue of nontreated and treated adult mice were spread on metal screen, air dried, shadowed with uranium and observed by electron microscope.

In untreated animals the ground substance appeared finely granular or homogeneous. Substances studied were hydrocortisone, growth hormone, desoxycorticosterone, heparin and hyaluronidase. Hydrocortisone induced specific striking regular granulation of ground substance not found for any other agent. Stressing intact but not adrenalectomized animals produced this specific change.

Ground substance of adrenalectomized group was agranular, homogeneous and cloudy. Similar appearances were produced in intact animals by desoxycorticosterone or hyaluronidase.

Uniformly scattered granules (80–100 Å) produced tufted appearance of ground substance following hydrocortisone treatment. Larger bodies (4,000 Å) were found in or/and on the matrix of ground substance in groups treated with corticosterone. No granules were found in the ground substance of adrenalectomized or intact mice treated with hyaluronidase. The relationship of these changes in ground substance to the antiphlogistic action of C-11 hydroxycorticosteroids is being studied.

191. *The fate of intravenously injected cells of Ehrlich's ascites tumor.*[1] Ralph N. BAILLIF, Department of Anatomy, Tulane University.

Peritoneal exudate from mice bearing the Ehrlich tumor is a rich suspension (80–150 $\times$ 10^6 cells/cm^3) of discrete, viable, actively mitotic cells. When heparinized, this suspension is suitable for intravenous injection; 0.1 cm^3 is tolerated by a 25-gm mouse if injected slowly.

During the first 12 days after the single inoculation, there is a generalized lymphatic, myeloid and reticulo-endothelial hypertrophy. It is probable that most of the injected cells either leave the blood stream by migrating into perivascular connective tissues or collect in sinusoids of the suprarenal, liver, spleen and bone marrow. A few mitotic figures appear in these locations, but many of the cells appear to be disintegrating. No evidence of embolism was found.

Beginning on post-inoculation days 10–12, microscopic pulmonary metastases can be found in almost every case, centered in the pulmonary stroma. These grow so rapidly that most of the hosts succumb during the third or fourth week. Small metastases also appear in sinusoids of the liver and adrenal, but these do not appear to be progressive.

It is obvious that by intravenous injection many tissues are placed in contact with viable tumor cells; notwithstanding, the sites for metastases are few. An explanation for this phenomenon may well be associated with differential susceptibility to tumor growth.

[1] Supported by a grant-in-aid from the United States Public Health Service.

49. The effect of hypophysectomy on the gastric mucosa. Burton L. BAKER and Gerald D. ABRAMS*, Department of Anatomy, University of Michigan.

Hypophysectomy of rats has little effect on the amount of mucus contained in the columnar epithelial cells of the pits and surface of the gastric mucosa. It increases the amount of mucus in the mucous neck cells. Many parietal cells are smaller, contain fewer mitochondria and usually fewer fuchsinophilic granules. The zymogenic cells undergo a profound involution accompanied by loss of pepsinogen granules and cytoplasmic basophilia (ribonucleic acid). Biochemical determination (colorimetric hemoglobin method of Glass, Pugh and Wolf) of the peptic activity of the gastric juice secreted during a 6 hour period after pyloric ligation shows that hypophysectomy causes a striking reduction in the capacity of zymogenic cells to secrete this enzyme. The involution of zymogenic cells and their reduced functional activity is present 3 days after the operation and reaches a low level at 7 days. Significant recovery was not observed during the 128 day period of study after hypophysectomy. It is concluded that the hypophysis exerts a regulatory influence over the activity of gastric zymogenic cells and other evidence at hand indicates that the pars distalis is primarily responsible.

182. Effects of ultrasound on the central nervous system.[1] John W. BARNARD, Ruth Baumann FRY*, Don TUCKER*, and William J. FRY*. Bioacoustics Laboratory, University of Illinois.

This is a continuation of the studies of the effects of focused, high intensity ultrasound (980 kilocycles) on the central nervous system. The areas selected for study were the motor cortex of the cat and of the monkey and the internal capsule and adjacent nuclei of the cat. These areas, after removal of the intervening bone, were irradiated with ultrasound of different intensities and for varying durations. These animals were sacrificed after various time intervals and the brains were studied with Nissl, Marchi and silver stains. The differences in the responses of the cells, fibers and blood vessels were especially observed in an effort to find the exact site at which ultrasound has its effects.

[1] Partially supported by the Physiology Branch of the Office of Naval Research.

6. *Influence of pituitary gonadotropin (FSH) on the metabolism of the ovary.*[1] Marion I. BARNHART*, Department of Physiology, Wayne University College of Medicine and Detroit Institute of Cancer Research. (Introduced by Gordon H. Scott.)

Ovaries of recently born mice were cultured according to the watchglass method. One member of each pair of heterologous plasma clots was fortified with Armour's Pituitary Gonadotropin (FSH) in concentrations varying from 0.05 to 0.50 Armour units per clot. On these matched clots were single representatives of the pairs of ovaries from each of several litter mates.

To determine if the hormone had an effect on the metabolism of these explanted ovaries, studies of their oxygen consumption were undertaken, using a Stern-Kirk type differential microrespirometer. A pool of as few as 5 ovaries consumed sufficient oxygen to be measured. Following equilibration, readings were taken at 10 to 15 minute intervals for an hour. At the conclusion of the run, the ovaries were measured and fixed for microscopic examination. Results were expressed in terms of microliters of oxygen consumed per hour, per mm^2 of surface at standard conditions. Histologically, the ovaries were normal.

Ovaries grown on unfortified plasma clots gave oxygen uptake values agreeing within 10% with values obtained from control ovaries grown on similar clots. Oxygen consumption of groups of ovaries grown on clots containing 0.1 to 0.3 units FSH was enhanced 20 to 30% when compared to values from groups of control ovaries cultivated on unfortified clots.

[1] Aided by an Institutional Grant from The American Cancer Society.

3. *Morphology of epidermal nuclei in hermaphrodites.*[1] Murray L. BARR, Department of Microscopic Anatomy, University of Western Ontario.

Epidermal nuclei of females contain a special mass of chromatin, the sex chromatin, which is seldom seen in nuclei of males. The sex chromatin of female nuclei is thought to be formed from heterochromatic regions of the two X chromosomes. The structure of epidermal nuclei, therefore, gives an indication of the chromosomal sex of an individual.

Skin biopsies from 30 hermaphrodites were studied. Seventeen patients had female-type nuclei. This group included three true hermaphrodites and 14 female pseudohermaphrodites (13 patients with adrenogenital syndrome and one with no evidence of adrenocortical hyperplasia). Thirteen patients had male-type nuclei. This group included one true hermaphrodite, one female pseudohermaphrodite (not adrenogenital syndrome), and 11 male pseudohermaphrodites.

This preliminary study suggests that the rare true hermaphrodites and female pseudohermaphrodites not caused by adrenocortical dysfunction have either female-type nuclei or male-type nuclei. Patients in the main group of female pseudohermaphroditism, caused by adrenal hyperplasia, have female-type nuclei. Male pseudohermaphrodites, on the other hand, have male-type nuclei. The skin biopsy test is a useful aid in differential diagnosis of the main groups of hermaphrodites, namely, patients with the congenital adrenogenital syndrome, and male pseudohermaphrodites.

[1] Supported by a grant from the National Research Council of Canada.

171. *Influence of testosterone on the ovary of prepuberal female mice.* Charles A. BARRACLOUGH*, Bureau of Biological Research, Rutgers University and Department of Anatomy, University of California at Los Angeles. (Introduced by Charles H. Sawyer.)

Female mice of 5, 10 and 20 days of age were given single subcutaneous injections of testosterone propionate (1.0 mg.) and compared with littermate controls at varied intervals up to 60 days of age. Ovarian alkaline phosphatase normally was present in the theca, nucleoli of the oocyte and nuclei of the interstitial tissue and granulosa cells at 10 days of age. At subsequent ages an increase in thecal enzyme was observed in contrast to an absence of enzyme in large vesicular follicles. The lutein cells of the corpora lutea revealed marked concentrations of enzyme. Interstitial lipid (neutral fat, phospholipid and cholesterol) was normally first observed at 10 and thecal lipid (neutral fat and phospholipid) at 20 days of age. While interstitial lipid and alkaline phosphatase were not affected by androgen, follicular atresia was apparent in animals treated at 5 days of age and autopsied at later ages. Concomitant with atresia was the appearance of alkaline phosphatase and sudanophilic lipid in the granulosa cells and oocytic cytoplasm. Corpora lutea, which normally appear at 40 days of age, were absent in mice treated at 5 but not in those injected at 20 days of age. Animals treated at 10 days assumed an intermediate position. Animal age at the time of hormone administration is a factor influencing results.

118. *The reaction of arterial elastic tissue to grafting.* William H. BATCHELOR* and James W. PATE*, Physiology Division, Naval Medical Research Institute. (Introduced by Sam L. Clark)

Aortic segments grafted into the dog display regularly a fragmentation of elastic tissue. This reaction does not interfere with satisfactory function of the graft for the first few years, but it deserves investigation in its own right as well as for guidance in the use of grafts.

Both fresh and freeze-dried aortic segments were grafted and subsequently removed from the site after a period ranging from one week to one year. Frozen sections of both fresh and formalin-fixed specimens were examined by phase contrast as well as by ordinary illumination using stains. Comparison was made with stained sections cut from paraffin-embedded specimens.

The medial elastic net characteristically shows widespread fragmentation, most marked subintimally. Adjacent host tissue shows a similar fragmentation, in contrast to specimens taken from aortas without grafts. The most severely affected regions show disruption of gross architecture. The individual fibers or plates show both transverse and longitudinal fragmentation yielding a curiously uniform pattern. The less severely involved regions, on the other hand, are difficult to distinguish from certain areas of control sections interpreted as fenestrated elastic, thought to be a normal feature.

The change in individual fibers invites comparison with elastase-treated fibers as described by Balò and Banga. Interpretation of these changes must begin with the recognition of involvement of host along with graft tissue.

167. A comparison and evaluation of procedures for the plasmal reaction.[1] W. D. BELT* and E. R. HAYES, Department of Anatomy, The Ohio State University.

Many variations of the original plasmal reaction of Feulgen and Voit have been practiced. The various published techniques are here compared upon uniform material and the influence of individual steps analyzed. The procedures differ mostly in the fixatives used, therefore formalin, formol-calcium, formol-sublimate, sublimate, sublimate-acetic, formol-acetic, copper sulphate, and heat were evaluated for their effect upon the reaction. In addition to unembedded tissues, tissues embedded in gelatin and polyethylene glycol were studied. The results show that small variables often exert a marked influence upon the reaction. The less the pretreatment (fixation) the more valid the result. To be considered valid, a reaction must display (1) an essentially negative control, (2) a distribution and intensity corresponding to the reaction upon fresh tissue and (3) reproducibility. A technique is presented for performing the reaction upon better preserved and somewhat thinner sections than usual. The unfixed sections are kept frozen until permitted to thaw in an atmosphere of formalin vapor. After a brief fixation they are washed and subjected to the reaction proper.

[1] This investigation was supported in part by a research grant C-1817 M and G from the National Cancer Institute, of the National Institute of Health, Public Health Service.

180. Location of hypothalamic lesions affecting the adenohypophysis. E. M. BOGDANOVE*, Departments of Anatomy, State University of Iowa and Albany Medical College. (Introduced by J. M. Wolfe)

Analysis of massive hypothalamic lesions resulting in various adenohypophysial dysfunctions (Bogdanove and Halmi, 1952, 1953) showed lesions interfering with thyrotrophin and gonadotrophin secretion to include the regions of the suprachiasmatic and arcuate nuclei, respectively. McCann likewise (Am. J. Physiol., *175*, 13, 1953) has found the arcuate nucleus consistently damaged in large lesions producing testicular atrophy.

To examine the possibility that these nuclei might mark the locations of discrete "centers" concerned with these specific endocrine functions, much smaller lesions were similarly placed in a series of 45 adult male rats which were subsequently given propylthiouracil for 18 days. Only one rat of this series showed severe, three others exhibiting partial, testicular atrophy. No unequivocal impairment of thyrotrophin secretion was found, although three rats had thyroid weights of 26 mg. (controls: 48–63 mg.).

Examination of these few effective small lesions showed that gonad atrophy followed lesions of the arcuate nuclei, while the (probable) impairment of thyrotrophin secretion was not related to suprachiasmatic nuclear damage. However, extensive destruction of both of these nuclei did occur unaccompanied by discernible endocrine effects.

More detailed description of these lesions will be presented, and the bearing of this study on the problem of hypothalamic localization discussed.

56. *On the reticular connections of structures in the vicinity of the chemoreceptor trigger zone for emesis.*[1] K. R. BRIZZEE, Anatomy Department, University of Utah College of Medicine.

Experiments have been carried out to determine the afferent reticular connections of certain structures adjacent to the chemoreceptor trigger zone for emesis (Wang and Borison, '50, '51) including the area postrema, nucleus gracilis, descending vestibular nucleus, and dorsal sensory vagal nucleus (ala cinerea). Following unilateral cauterization of the nucleus gracilis numerous chromatolytic neurons were seen in the central region of the inferior reticular nucleus. These alterations were mainly ipsilateral but a few altered cells were found in the contralateral nucleus. Unilateral cauterization of the area postrema and ala cinerea simultaneously resulted in a few chromatolytic neurons in the same area of the inferior reticular nucleus ipsilaterally and also in the dorsal and ventral portions of the paramedian reticular nucleus and the dorso-lateral portion of the reticular formation in the region of the vomiting center (Wang and Borison, '50) on the same side.

No alterations in the reticular nuclei were observed following lesions restricted to the main body or superficial portion of the area postrema.

[1] Supported by a grant from the U. S. Public Health Service.

106. *The relationship of age to the amount of pigment deposition in cortical nerve cells.* Harold BRODY*, Department of Anatomy, University of North Dakota. (Introduced by Christopher J. Hamre)

In the program of studies upon quantitative changes in the cerebral cortex, examination was made of pigment deposition within the cortical nerve cells. The purpose of the study was to compare the pigment content of cells within different cortical areas and its presence in a human series from the newborn to ninety-five years of age. Cases were chosen which did not demonstrate any known neurological disorder prior to death.

Sections were stained by the periodic acid-Schiff technique which was found to demonstrate most clearly these pigment granules. Cells examined were from brains in four age categories: newborn, twenty years, forty-five years and over seventy years of age. One hundred cells in layer three were examined in each specimen and percentages computed on the basis of pigment content of these cells.

The precentral gyrus, postcentral gyrus and superior temporal gyrus follow a similar pattern in regard to the number of cells containing the pigment granules and the amount of pigmentation in each cell. Fewer cells contain pigment in the striate area than in the other three gyri examined.

In all areas a direct relationship exists between the number of cells containing pigment granules and the age of the individual specimen. As the age increases, the number of cells involved and the degree of involvement increases.

84. Quantitative cellular changes in the bone marrow of albino rats resulting from protein deficiency. Jerry W. BROWN*, Department of Anatomy, University of Pittsburgh. (Introduced by Davenport Hooker)

A quantitative cellular study was made of bone marrow from male albino rats fed a 0% protein diet beginning at 31 days, their pair fed controls (from 32 days) and a normal group (from 31 days) fed a normal 18% protein diet. The tests lasted 40 to 41 days. Femoral bone marrow, fixed in Zenker-formol, was paraffin embedded, serially sectioned at 8 μ and stained with hematoxylin-eosin-azure II. Differential cell counts were made in areas of 100 μ^2 in 12 bone marrow sections from each rat, the mean number of cells per mm^3 being computed.

Results show that the number of erythroblasts and of erythroid cells in mitosis in marrow of the 0% protein group decreased in the same proportion as that from pair fed controls. However, protein deficency caused a greater reduction in normoblast population. The latter change appears to be primarily responsible for the accompanying peripheral anemia. Protein deficiency per se had little effect on the populations of early and late forms of eosinophils and heterophils, but did result in a greater increase in the number of heterophil cells in mitosis. Protein inanition reduced the number of megakaryocytes and basophils. Furthermore, protein deficiency decreased the plasma cell population much more than did general inanition.

137. *Radial and ulnar arterial anastomosis in the hand.* Henry C. BROWNING, Department of Anatomy, University of Puerto Rico, and David E. MORTON, Department of Anatomy, Yale University.

Five types of anastomosis between the superficial and deep arterial arches were found in 100 hands: ulnar artery and deep arch; common digital and volar metacarpal arteries; princeps pollicis artery and superficial arch; superficial volar radial artery and superficial arch; and common digital and dorsal metacarpal arteries. The commonest pattern included the first three or first four of these anastomoses.

The most constant branches were connections between the ulnar artery and deep arch, the princeps pollicis artery and, less so, the radial index artery. Commonly the adjacent sides of the second and third digits were supplied by the radial index artery rather than by the common digital artery. Frequently adjacent sides of other digits received an analogous supply.

The dorsal metacarpal arteries, except the second, were usually small and relatively unimportant. The deep arch was usually smaller than the superficial but the radial and ulnar arteries were of similar size. An isolated supply of the first and second digits by the radial artery was rare.

108. A comparative quantitative analysis of perineuronal satellite cells in motor cortex. Robert H. BROWNSON*, Department of Anatomy, University of Southern California School of Medicine. (Introduced by Paul R. Patek)

In an earlier investigation (Brownson, 1953, unpublished) it was noted that some definite signs of age changes in glial satellite cell relationship between young

and old mice was evident in the spinal cord, anterior horn cells. Also because of variations reported by earlier investigators on the morphologic alteration and function of perineuronal satellite cells in apparently normal, aging nervous tissues, the present author feels justified in furthering this investigation. No attempt to discuss the physiology is involved, but rather the comparative anatomical relationship between large neurons and glial satellites in the motor cortex of mouse, rat, and human brains is undertaken.

A method for determining the numbers of glial satellite cells surrounding each neuron on a given plane at a fixed distance gives a quantitative estimation of glial cell relationship per unit area of nervous tissue for different age groups.

Microscopic examination performed with a binocular microscope and micrometer disk revealed a marked decrease in perineuronal satellite cells with increase in age. Young mice showed 56–58 percent satellitosis while 32–40 percent satellitosis characterized the senile mice. Young immature rats showed 72–76 percent satellitosis while markedly senile rats accounted for only 32–50 percent satellitosis. Human specimens are at present under investigation.

13. *Electroarchitectonic mapping of afferent and "antidromic" potentials in the cerebral cortex.* Nathaniel A. BUCHWALD*, Department of Anatomy, Tulane University. (Introduced by Harold Cummins)

Intracortical potentials of cats were investigated by electroarchitectonic mapping. Potentials evoked by electrical stimulation of the sciatic nerve complex or the dorsal funiculus and by "antidromic" volleys fired into the lateral cortico-spinal tract or into the medullary pyramids were detected by a penetrating electrode from stations at and beneath the cortical surface. Potentials evoked by afferent stimulation of the sciatic complex or dorsal funiculus were recorded at the cortical surface as biphasic waves. The initial, positive, phase had a duration of 8–12 milliseconds. At a depth of 1 + mm beneath the cortical surface this positive phase was analyzed into two components, an early "non-labile" portion and a "labile" portion which reversed to negativity.

"Antidromic" volleys into the lateral cortico-spinal tract or the medullary pyramids evoked, at the cortical surface, a brief diphasic positive-negative potential. Its first phase did not reverse when the electrode penetrated beneath the cortical surface.

Although potentials evoked by "antidromic" and by afferent stimulation could be detected at similar loci in the post-cruciate cortex they were distinguishable by (1) shorter latency and duration of the "antidromics," (2) differential response to topical anesthetization and (3) differences in sign, form and intensity when recorded beneath the surface.

Electroarchitectonic maps illustrating the depth-time-voltage relationships of these potentials will be shown and the observations will be discussed with regard to possible cortical function.

130. Transplantable and transmissable mouse tumors which cause growth stimulating effects on sympathetic and spinal ganglia of the embryonic chick.[1] Elmer D. BUEKER, Department of Anatomy, University of Missouri.

After intraembryonic transplantation, mouse sarcomas (I, 37, 180) cause excessive growth of chick spinal and sympathetic ganglia. Sarcomas (37, 180) stimulate growth after implantation on the allantois or when cultured in vitro with ganglia (Levi-Montalcini, '52, '53). Mouse tumors (sarcomas: T241 Lewis, Ma387, A274, S635, MCI, Ds4; carcinomas: C954, Ca15) were studied for nerve growth effects. Tumors were incorporated unilaterally in hind limb fields of 2½-day chicks. From 700 experiments 325 specimens were obtained, sacrificed 6–16 days' incubation and prepared with De Castro's modification of Cajal's technique and Wenger's hematoxylin. Nerves and ganglia related to tumors were identified from graphic reconstructions. Quantitative data were obtained from cardboard reconstructions of ganglia, one case for each series. Total volume of lumbosacral ganglia which supplied nerves to sarcoma T241 Lewis, in the case selected, was 1.74 × greater than the contralateral; sympathetic ganglia 1.82 ×. Volumes of spinal ganglia of Ma387, A274, S365, MCI and Ds4 were 1.71 ×, 1.33 ×, 1.51 ×, 1.25 × and 1.15 × greater respectively; sympathetic ganglia: 1.34 ×, 1.19 ×, 1.09 ×, 1.20 ×, 1.08 ×. Experimental spinal ganglia of cases Ca15 and C945 were 0.92 × and 0.86 × the control; sympathetic 0.95 × and 0.89 ×. Nerve growth stimulating properties seem widely distributed in mouse sarcomas and vary only in intensity. Carcinomas are entirely negative.

[1] Aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health.

76. Cytometric effects of male sex hormone on rat seminal vesicles.[1] L. F. CAVAZOS* and R. M. MELAMPY, Agricultural Experiment Station, Iowa State College.

A cytometric study was made of the effects of castration and hormone replacement on the secretory epithelium of seminal vesicles of sexually mature rats. Average diameters of equivalent circular cross sections of nuclei were calculated from nuclear areas obtained by camera lucida. Cell heights were measured with an ocular micrometer. Twenty-four hours following gonadectomy the nuclear diameter decreased 0.1 μ (not Sig.) and cell height 4 μ ($P = 0.01$). The diameter was reduced 0.5 μ ($P = 0.10$) and the height 8 μ ($P = 0.01$) in 60 hours after castration. Nuclear reduction of 1 μ ($P = 0.01$) and cell height of 11 μ ($P = 0.01$) occurred during a 38-day period. In the case of hormone-treated animals (500 μg testosterone propionate daily) the diameter increased 0.7 μ ($P = 0.01$) and the cell height 1 μ ($P = 0.10$) in 12 hours. By 60 hours the diameter increased 2 μ ($P = 0.01$) and the height 9 μ ($P = 0.01$) above the values for 38-day castrates. After this the nuclear diameter decreased whereas the cell height remained relatively constant. Mitoses were not visible in the epithelium of castrates, or the 24- and 36-hour treated animals. A mitotic activity of 7.5% was noted after 60 hours. Following this a sharp decline occurred, and in animals injected 20 days a value of 0.1% was observed.

[1] Supported by grant C-1779, National Cancer Institute of the National Institutes of Health.

68. *Vascular nerves and cholinesterase of blood vessel walls.*[1] Kermit CHRISTENSEN and Therese STUESSE*, Department of Anatomy, St. Louis University School of Medicine.

Various interrelationships between the finer vascular nerves and neurohumoral mechanisms active in the walls of blood vessels have been postulated. One phase of this problem is being studied by comparing sections of blood vessels of the cat prepared by a modified Bielschowsky silver technique to show the terminal nervous network, with sections of the same blood vessels in which cholinesterase has been demonstrated histochemically by the long-chained fatty acid ester technique or by the thiocholine technique. The results of the histochemical techniques have been checked each time by subjecting sympathetic ganglia to the same procedures as the blood vessels.

In arteries cholinesterase is localized chiefly in the media. Its appearance as coarse granules scattered throughout the media, as somewhat smaller granules sometimes limited to the periphery of the media, or as a non-granular brownish gray component of the media is determined in part by the pH of the substrate solution. Individual variations in amount of cholinesterase of the same arteries, and the variations in amount in arteries of different sizes in the same individual appear to correspond with determinations that have been made by other methods.

In the silver preparations, the nervous network is localized in the adventitia. The portion of the net which is close to the media has been suggested to be efferent in function. In the preparations studied, the boundary of the adventitia with the media is the important site where the terminal nervous tissues come into contact with tissues containing the enzyme.

[1] Aided by a grant from the Public Health Service (B-497 C5).

177. *Morphology and histochemistry of a vermiform nerve end organ hitherto undescribed in mouse dermis.* C. H. U. CHU and C. A. SWINYARD, Department of Anatomy, University of Utah College of Medicine.

Whole mounts of mouse dermis separated by immersion in one per cent formic acid reveal a vermiform structure which, when stained with Holmes', Romanes' and Bodian's methods, proves to be a nerve end organ.

Each end organ is encapsulated by a single layer of cells. Within the capsule is a thick layer of "granular substance," in which the myelin is dispersed in granular form. Aniline acid fuchsin stains the entire "granular substance" a diffuse red color. The axis cylinder of the nerve fiber continues into the "granular substance" occasionally terminating in a knob.

Many histochemical tests have been performed to determine the composition of the end organ. Since the tissue has been treated with one per cent formic acid which might affect the solubility or chemical nature of a certain compound, a negative reaction does not necessarily mean its absence. However, only positive results indicating 1,2-glycol, unsaturated fatty acids, amino acids and others will be presented and discussed.

9. *Cerebellar stimulation in unanesthetized fish.* Sam L. CLARK, Department of Anatomy, Vanderbilt University.

Preliminary experiments demonstrated the feasibility of stimulating the cerebellum of the unanesthetized gold fish (6 to 7 inches in length) by means of a small implanted electrode built of balsa or plastic with light insulated wire attached. Fish were stimulated while swimming freely in a porcelain sink in water about five inches deep. Sufficient free wire allowed the fish to reach any portion of the pool. Stimulation was done with a constant current stimulator (Montgomery, L. H. and J. W. Ward, 1951) and effects were obtained with less than 1 ma. Results of stimulation were observed as changes in direction or spontaneous spurts of swimming, associated with fin and eye movements. The pattern varied with the point on the cerebellum that was stimulated. Stimulus of posterio-lateral areas was accompanied by circling or spiralling homolaterally, followed on cessation of the stimulus by rebound movements tending to be mirrored images of the phase of stimulus. These resembled in pattern part of the responses elicited from the cerebellum of the cat with implanted electrodes (Clark, 1939). Thus far no long after-effect comparable to that seen in the cat has been observed.

85. *A possible role of the polymorphonuclear leukocyte in the production of fever in the rabbit by injections of bacterial pyrogens or antigenic proteins.* Sam L. CLARK, Jr.*, Physiology Division, Naval Medical Research Institute. (Introduced by R. S. Farr)

A transient, profound granulocytopenia precedes the fever produced by an intravenous injection of bacterial pyrogen. A comparable picture may be produced by injections of antigenic proteins. Thus, Farr (Abstract submitted to Am. Assn. Anat., 1954) has shown that those rabbits which develop antibodies in response to injections of bovine albumin, eventually respond to the injections with fever; and we find that a granulocytopenia precedes this fever also.

An effect of these substances on the granulocyte can be demonstrated *in vitro.* Incubation of the agents with rabbit blood produces vacuolization and disintegration of the heterophil leukocytes. The cells were observed in wet preparations by phase microscopy and compared with suitable controls. The conditions necessary to elicit this effect suggest that antibody is required for the action of protein antigen, and, similarly, that humoral factors operate in the pyrogen system.

These findings suggest that a common mechanism involved in the production of fever after injections of bacterial pyrogens, or proteins in sensitized rabbits, may be lysis of granulocytes and the release of a fever-producing substance, such as that described by Bennett and Beeson (1953).

161. *The perforatorium, an extension of the nuclear membrane of the head of the rat spermatozoon.* Y. CLERMONT*, Department of Anatomy, McGill University. (Introduced by C. P. Leblond)

By staining techniques and electron microscopy, it is possible to differentiate the acrosome and perforatorium — two structures which, together with nucleus and head cap, compose the head of the rat spermatozoon. The acrosome — a rod-like structure embedded in the head cap — is independent of the nucleus. The per-

foratorium, on the other hand, is closely associated with the apex of the nucleus and extends beyond it as a narrow, tapering pyramid.

To determine the relationship between perforatorium and nucleus, suspensions of spermatozoa treated with a 1N sodium hydroxide solution were examined by phase contrast microscopy. Under these conditions, the acrosome and head cap dissolved rapidly, the nucleus more slowly, and the nuclear membrane and the perforatorium persisted. Thus, the last two structures were found to be continuous. Furthermore, the three prongs by which the perforatorium is attached to the nuclear apex could be identified as thickenings of the nuclear membrane.

It is suggested that the perforatorium is an outgrowth of the nuclear membrane arising as a result of the changes taking place in the head of the spermatid during the latter part of spermiogenesis.

163. Histochemical observations on the Harderian gland of the albino mouse. Sidney A. COHN*, Division of Anatomy, University of Tennessee. (Introduced by Laurence R. Fitzgerald)

The Harderian gland is a compound tubulo-alveolar, apocrine gland situated behind the eyeball. The glandular epithelium consists of a single row of pyramidal cells resting on a basement membrane. These cells are filled with sudanophilic droplets. Aggregated droplets together with some of the apical portion of the cells are extruded in the form of globules. The coalescence of several globules within a lumen forms a large sudanophilic mass within which the identity of the droplets is retained. In addition to its lipid secretion the gland usually contains a characteristic porphyrin-pigment in several of its alveolar lumina.

Glycogen is not demonstrable in the mature gland with the periodic acid-Schiff technic. There is, however, an unknown material in many lumina which recolors the Schiff reagent; this material shows great variability in amount, intensity of coloration and distribution. There is a negative correlation between the amount of this Schiff-reactive material and the amount of pigment. Pigment is rarely found except in those lumina that also contain the Schiff-reactive substance. A relationship may exist between this Schiff-reactive material and the formation of the porphyrin-pigment.

56. Subcutaneous suspensory mechanism of penis and scrotum in 55 series of gross sections, photographically recorded. Also the suspensory penile ligament (BNA). E. D. CONGDON and J. M. ESSENBERG, Department of Anatomy, The Chicago Medical School.

A subcutaneous suspensory mechanism of penis and scrotum was photographed in over 50 series of sections in three planes. It is formed of connecting fibrous sheets or masses imbedded in fatty areolar tissue. Its central part is made up almost entirely of fine elastic fibers. It changes continuously in pattern when traced caudally from the level of the anterior superior iliac spines to the penis and scrotum. Its structure varies with the thickness of the subcutaneous layer. It has a wide dorsal attachment to the musculo-skeletal layer of the body wall.

The segment of the mechanism cranial to the superficial inguinal rings is oval in transverse section or, if connected with the superficial fascia, spindle shaped.

It is formed largely of a net of sheets. Its intermediate division between the rings and penis and scrotum has a dense central body triangular in transverse section and a system of sheets extending laterally to form gutters for the spermatic cords. The medial origin of these sheets from the central part is continued caudally onto the dorsum of the penis. The cranio-caudal extent of origin and of it and its gutters is more than 5 cm. These gutters open on their medial side, were distorted in dissection by McGregor to form his "third inguinal rings."

The suspensory penile ligament (BNA) which lies deep to and largely independent from the subcutaneous mechanism is a three dimensional net of fibrous bands several millimeters in thickness.

88. *Mast cells and human atherosclerosis.*[1] Paris CONSTANTINIDES and Alexander CAIRNS*, Department of Anatomy, University of British Columbia.

Mast cells were counted in toluidine blue stained paraffin sections from the formalin fixed myocardium of 46 young adults without evidence of atherosclerosis, 48 senile adults with marked generalized atherosclerosis, and 48 senile adults without evidence of atherosclerosis. The three groups were evenly divided in males and females. Evaluation of the heart mast cell counts of these mixed populations showed that: (1) Atherosclerotic seniles had a lower count than non-atherosclerotic young adults (p less than 0.001). (2) There was no significant difference between the counts of non-atherosclerotic seniles and non-atherosclerotic young adults. Comparison of male and female counts revealed that: (1) Among the non-atherosclerotic young adults, the males had a lower count than the females (p between 0.02 and 0.01). (2) Among the females, atherosclerotic seniles had a lower count than non-atherosclerotic young adults (p less than 0.001). (3) Among the females, there was no significant difference between the counts of non-atherosclerotic seniles and non-atherosclerotic young adults. (4) There were no significant differences between the counts of the males in the three groups.

These findings will be discussed in the light of some theories concerning the pathogenesis of atherosclerosis.

[1] Supported by the National Research Council of Canada.

93. *The pituitary erythropoietic factor.*[1] A. N. CONTOPOULOS*, M. E. SIMPSON, D. C. VAN DYKE*, S. ELLIS*, J. H. LAWRENCE* and H. M. EVANS, Institute of Experimental Biology and Donner Laboratory, University of California, Berkeley.

Removal of the pituitary results in a severe and progressive anemia with approximately 50 per cent decrease in hematocrit, hemoglobin and red cell volume. Ablation of the thyroid, adrenals or testes, alone or in combination, results only in a mild anemia. Administration of the target organ hormones, singly or in combination, does not repair the post-hypophysectomy anemia, although these hormones repair the anemia resulting from their respective deficiency states. However, administration of pituitary extracts results in repair of post-hypophysectomy anemia as judged by red blood cell counts, hemoglobin, hematocrit and red cell volume determinations. The stimulation of erythropoiesis is also shown by an in-

creased number of circulating reticulocytes and normoblasts. Evidence is presented that this pituitary erythropoietic factor does not stimulate erythropoiesis through any of the known pituitary target organs since it stimulates red cell production in the absence of the pituitary and target organs. This factor when given orally or parenterally to the normal rat produces polycythemia.

[1] Supported by U. S. Public Health Grant A-366 and the Atomic Energy Commission.

39. *Effects of reduced blood supply on the chemistry and histology of bone.*[1] Carl C. COOLBAUGH, Department of Anatomy, Wayne University. (Introduced by F. Gaynor Evans)

Surgical reductions in the blood supply to bone compacta have been shown to cause an initial postoperative interval in which the density and modulus of elasticity are reduced; and a secondary postoperative interval in which they are both increased.

Previous chemical analyses of the inorganic constituents of the bone have been correlated with analyses using Ca^{45} and P^{32} in an attempt to further relate them to the changes noted in the physical properties of the bone.

[1] Supported by a research contract with the National Arthritis and Rheumatism Foundation.

97. *Alloxan inactivation of coenzyme A and the liver acetylating enzyme.* S. J. COOPERSTEIN and Arnold LAZAROW, Department of Anatomy, Western Reserve University.

In vitro studies were carried out in which either the acetylating enzyme or the coenzyme A was preincubated with alloxan (.002M to .000025M) at pH 7.4 for 60 minutes. The other components of the system (Kaplan and Lipman) were then added and the rate of sulfonamide acetylation was measured in the presence and absence of added cysteine. In control experiments the alloxan was preincubated in buffer (pH 7.4) and the decomposition product (alloxanic acid) was then added to the test system.

Preincubation with .0005M alloxan inactivated 70% of the acetylating enzyme and 30% of the coenzyme A (acetylation measured in the absence of added cysteine). Since the probable maximum *in vivo* concentration of alloxan, following the intravenous injection of 40 mg/kg is in the neighborhood of .0004M (assuming that the diabetogenic dose is uniformly distributed throughout body water), the observed enzyme inactivation may be of physiological importance. Significant *in vitro* inactivation of other enzymes (hexokinase, phosphohexokinase, phosphoglucomutase and succinic dehydrogenase) has been observed previously only at alloxan concentrations about five times greater than the probable maximal *in vivo* alloxan concentration.

Although the significance of the acetylating enzyme is not known, these results suggest that the diabetogenic mechanism of alloxan may be related to its reaction with sulfhydryl enzymes. Similar enzyme studies will be undertaken, using the isolated islet tissue of fish.

100. Anatomical factors affecting the physical resolution of directional counters used in I^{131} tracer studies of human thyroid glands.[1] P. L. COX* and L. G. STEVENS-NEWSHAM*, Departments of Anatomy and Radiology, McGill University. (Introduced by Professor C. P. Martin)

In vivo determinations of the overall mass and finer structure of a human thyroid gland by I^{131} tracer studies depends not only on the characteristics of the isotope and detection apparatus used but also on anatomical and physiological variables within the gland itself.

To study such variables, a hollow plastic model of a human neck was filled with a set of cervical vertebrae, a rubber model of a trachea and larynx, a hollow plastic model of the thyroid gland of an anatomical subject, and water. The model of the thyroid was filled with a graded series of concentrations of isotope, either as a simple solution or enclosed in a series of glass or plastic spheroids similar to the follicles of a normal gland. Such variables as the effect of gland diameters, distance from detector, size, distance apart and differential in concentration between spheroids and general isotope concentration in the gland were determined by making a series of standard measurements with three types of counter, namely: beta, gamma and scintillation.

From such measurements the usefulness and errors inherent in the use of such counters will be discussed as well as their application to typical human tracer studies.

[1] Supported by The National Cancer Institute of Canada.

4. Action potentials in tissue cultures of chick embryo spinal ganglia.[1] Stanley M. CRAIN*, Department of Neurology, College of Physicians and Surgeons, Columbia University. (Introduced by Fred A. Mettler)

Action potentials in response to electrical stimulation have been obtained from chick embryo spinal ganglia after being cultured by the Maximow slide method for as long as two months. Intracellular as well as extracellular recordings have been made via electrolyte-filled glass pipettes whose tip diameters were much less than 1 micron. The membrane resting potentials of these ganglion cells ranged from 40 to 65 mV., and the evoked action potentials, 50 to 95 mV. The responses began after latencies of 0.3 to 12 msec. The spike was usually preceded by a slowly rising "prepotential" and followed by a long phase of hyperpolarization. The duration of these action potentials, excluding the last period, varied from about 2 to 5 msec. Many interesting potential patterns have been observed as a function of the stimulating and recording relationships, and also during the process of cellular deterioration following impalement. In some cases, intracellular responses could be obtained as long as 30 minutes after penetration, but usually both resting and action potentials either were seriously distorted or disappeared within a few minutes. Since the experimental arrangement permits simultaneous visual observation with a high powered microscope, the potentialities for detailed correlation of structure with function are quite promising.

[1] Supported by research grant no. B-105 from the National Institute of Mental Health of the National Institutes of Health, Public Health Service.

168. *Permeability of the rabbit placenta to glucose and fructose.* Jack DAVIES, Department of Anatomy, State University of Iowa.

The placental transfer of glucose and fructose in sixty rabbits has been studied from the fifteenth to the last day of gestation in normal conditions and following the parenteral administration of controlled amounts of these sugars. The fetal blood and also the amniotic and allantoic fluids normally contain 50 to 90 mg % of glucose and small amounts of fructose (3–6 mg %). The latter was estimated by Roe's method and has also been identified chromatographically.

Fructose has been found to penetrate with difficulty into the fetal fluids in marked contrast with glucose. Fetal blood levels of fructose are also low compared with those following the injection of glucose. There is evidence that some of the injected glucose is transformed into fructose during placental passage.

The possible causes underlying this differential permeability of the placenta to the simple hexoses, glucose and fructose, will be discussed in terms of the mechanism of placental transfer and in relation to the general problem of the fetal fructose which has remained a biological enigma since the time of Claude Bernard. Histochemical studies having a possible bearing on this problem, notably of alkaline phosphatase and of glycogen, will also be presented.

174. *The effects of Aminopterin (4-pteroylglutamic acid) and estrogen on the phosphoprotein phosphatase of the rat uterus.* James S. DAVIS*, Division of Anatomy, University of Tennessee. (Introduced by S. R. Bruesch)

Previous work has shown that Aminopterin and estrogen, when administered simultaneously to castrate weanling female rats, cause a striking increase in the incorporation of radioactive phosphorus into the phosphoprotein of the uterus, as compared to uteri treated with estrogen alone. In an attempt to relate this observation to an effect of Aminopterin on some specific enzyme system, no correlation could be found between activities of acid and alkaline phosphatase and the turnover of phosphoprotein phosphorus.

In the work to be reported uteri treated as above were assayed for phosphoprotein phosphatase activity by Norberg's method (Acta. Chem. Scand., 4: 1206–15, 1950). Preliminary experiments indicate that Aminopterin causes little change in the activity of this enzyme from which it might be concluded tentatively that this enzyme is not directly concerned in the turnover and synthesis of phosphoprotein. These experiments also indicate that the activity of this enzyme is somewhat higher in the uterus of the weanling rat than in that of the adult. The results will be discussed in relation to uterine growth.

175. *Instantaneous axes of rotation of the major extremity joints.*[1] Wilfrid T. DEMPSTER, Department of Anatomy, University of Michigan.

The wrist, ankle, elbow, knee, humeroscapular, and hip joints of cadavers have been analyzed kinematically to determine the location of the effective centers of joint rotation. Joint contacts are not strictly congruent; for one angular position certain areas of male and female surfaces are in contact, but for another position the regions of contact change. In joints of one degree of freedom there may be three or more contact areas for a given joint angulation. The number, position,

shape, and area of contacts change with rotation angle, so that each new contact is freshly lubricated with synovia. Although joint surfaces slide smoothly, the instantaneous axis of rotation for any joint follows a distinctive path as the joint is moved through its range of angular positions. Accordingly, no joint has fixed axes like pin-centered mechanisms. Instead the effective joint center shifts its location from instant to instant with different angular positions of the joint. Considerable variability in axis movement has been found from specimen to specimen for the same joint. The areas of contact for different joint angles as well as the curvature of articular contours and the paths of instantaneous centers for various angular positions will be illustrated.

[1] Supported by a contract with the Aero Medical Laboratory, Wright Air Development Center, Wright-Patterson A. F. Base, Ohio.

126. Observations on iodine metabolism in embryos of the terrestrial salamander Aneides anaeus. J. N. DENT,[1] Biology Division, Oak Ridge National Laboratory.

The development of numerous specimens of the bronzed salamander, *Aneides anaeus*, was followed from early limbbud stages throughout hatching and gill resorption. Its developmental morphology was found to differ from that previously described for another entirely terrestrial Plethodontid salamander, *Plethodon cinereus* (Dent, '42), only in minor detail. On the basis of histological observations, Dent ('42) concluded that the thyroid gland of *P. cinereus* functions only during the final stages of development. Autoradiographic preparations made from embryos of *A. anaeus* which had been immersed in solutions containing I^{131} showed that their thyroids begin to fix iodine near the end of the "intraoval" period and shortly before their gills reach maximal growth. The amount of iodine fixed in the thyroid then increases greatly as the gills regress and hatching ensues. No very marked localizations of iodine were found outside the thyroid; however, some iodine was retained in the developing skin and in the yolk of all stages studied and in the eye after the beginning of thyroid function. Destruction of the thyroid by immersion in solutions containing I^{131} at the stage of maximum gill extension did not prevent these animals from passing through the final stages of development.

[1] On leave from The University of Virginia.

109. A submicroscopic vesicular component in nerve satellite cells and in Schwann cells. Eduardo D. P. DE ROBERTIS and H. Stanley BENNETT, Department of Anatomy, University of Washington.

Electron micrographs of sections of buffered osmic fixed sympathetic ganglia of bullfrog and leopard frog revealed numerous capillaries, Schwann cells, and satellite cells attendant on ganglion cells. In confirmation of Palade, the capillary endothelial cells showed numerous vesicles, 400–600 Å in diameter, most abundantly in layers immediately adjacent to inner and outer capillary cell surfaces. Many of these vesicles communicated with endo- or pericapillary spaces by stomata which perforated the endothelial cell wall. Similar vesicles were encountered in Schwann cells and in satellite cells of sympathetic ganglia. These

vesicles were similar in size to those in capillaries, and some were observed in like manner to communicate with intercellular space through tubule-like stomata or openings. Being receptive to Palade's suggestion that the submicroscopic vesicles in capillary endothelium may represent fluid in transport by the process of pinocytosis on a submicroscopic scale, we postulate a similar behavior by the Schwann cells and satellite cells.

179. *Some afferent connections of specific nuclear masses of the hypothalamus.*[1] Gino DI VIRGILIO*, Department of Anatomy, University of Ottawa. (Introduced by J. Auer)

With the aid of the Horsley-Clarke stereotoxic apparatus, localized lesions were placed in 32 cats in the region of the septum, preoptic area, caudate nucleus and internal capsule, midline, supraoptic area, lateral thalamic nuclei, midline nuclei of the thalamus and dorsomedial thalamic nucleus.

The bouton technique confirmed the following tracts: septo-paraventricular, thalamo-paraventricular from dorsomedial thalamic nucleus, thalamo-periventricular from the midline nuclei of the thalamus, medullary-paraventricular from dorsolaterally placed structures; commissural fibers to supraoptic, suprachiasmatic and ventromedial hypothalamic nuclei, and a thalamo-infundibular tract to infundibular area.

Incidental to the above project, cortico-thalamic fibers were observed to reach the principal midline nuclei of the thalamus, the nucleus centro-median and the contralateral anterior thalamic nuclei.

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[1] Supported by a grant from the National Research Council of Canada.

52. *Observations on neurosecretion in the hypothalamo-hypophyseal system in a case of diabetes insipidus in man.* Glenn A. DRAGER and Glenn V. RUSSELL*, Department of Anatomy, The University of Texas Medical Branch.

The patient, a 43 year old white male, was admitted to the neurological service of John Sealy Hospital in April, 1953 after having had several "strokes" beginning in January, 1953. Among other neurological findings it was noted that he consumed and voided eight to ten liters of water per day. Urine concentration test showed specific gravity of 1.0096, confirming earlier suggestions of diabetes insipidus. Patient expired in September, 1953 and the hypothalamus and hypophysis were secured at autopsy.

Gross examination revealed numerous encephalomalacic areas resulting from vascular accidents, marked dilation of third ventricle, and enlargement of the median eminence. Sections of the hypophysis showed disorganization of cytological elements and absence of neurosecretion in the posterior lobe (azocarmine and Gomori stains). Serial sections of the hypothalamus showed a vascular accident had severed the upper area of infundibular stalk resulting in slight retrograde changes in the supraoptic and paraventricular nuclei, loss of neurosecretion in these nuclei, and in the hypothalamo-hypophyseal pathway (Nissl, myelin, and Gomori stains) although neurosecretory material can be routinely demonstrated in unaffected autopsy brains.

Results in a second case of diabetes insipidus which has recently become available will be reported.

185. *Additional observations on hyperstaining myelin and alterations of neuroglia induced by restricting the growth of the spinal cord.* Donald DUNCAN, Department of Anatomy, The University of Texas Medical Branch.

The author has previously reported that local restriction of spinal cord growth in young rats by ligature may cause changes in staining properties of myelin accompanied by increased numbers of supporting cells resembling those of neurilemma. The alterations were found in a group of rats with ligatures placed in the upper lumbar region. In this group extensive cavities above and below the ligature and functional impairment of the hind quarters suggested that trauma at the time the ligatures were applied might be a cause of the hyperstaining myelin and the neuroglial alterations. Consequently, in another group of rats ligatures were placed at the second cervical level with very little or no damage to the spinal cord at the time of operation. In this series no cavitation of the cord was produced and the animals were without symptoms between the time of operation and sacrifice three months later; however, the changes in stainability of myelin and the appearance of the supporting cells are the same as in the previous series. It is concluded that the modifications produced in the structure of the spinal white matter are not a result of initial trauma to the spinal cord, not chance findings unrelated to the experiment, but reproducible changes developing as a result of preventing the normal increase in diameter of the growing spinal cord.

120. *Membranous connective tissue.* Hans ELIAS, Department of Anatomy, The Chicago Medical School.

Fibers, when sectioned, appear as dots or short lines. If a fiber is to appear as a long line, it must be located in its entirety within the slice. This occurs very seldom. Thus, if van Gieson positive lines are abundant in sections, the presence of collagenous membranes in the tissue must be postulated.

Collagenous membranes occur in the human liver in cirrhosis; in the zona glomerulosa of the normal, human adrenal cortex; in the normal, human mammary gland and in the degenerated pancreas of dogs after ligation of the duct.

Fibrous and membranous collagenous tissue may be mixed; or one kind may be present to the exclusion of the other.

Collagenous membranes are frequently suspended in the meshes of reticular fiber networks, much as soap films in a net after dipping it into soap solution.

When the patterns of argyrophile and van Gieson positive material are identical as in the adrenal capsule, it is suggested that argyrophile fibers are coated with cylindrical films of collagen.

Where membranous collagen is found, fibroblasts are rare or absent. Membranous collagen seems to arise as a product of condensation of ground substance or tissue fluid. Some of this may be plasma which has seeped out of capillaries. Decomposition products of necrotic cells may also play a role in the genesis of collagen membranes.

53. Luteotrophin secretion invoked and maintained in female rat hypophyses by transplantation to the renal capsule. John W. EVERETT, Department of Anatomy, Duke University School of Medicine.

Adult female rats on the day after ovulation were hypophysectomized parapharyngeally and the pars distalis implanted in the left renal capsule. In series I the uteri were traumatized 4 days later and the subjects were autopsied on day 8. Completeness of hypophysectomy was established histologically. Results: large deciduomata in all engrafted animals (6 completely hypophysectomized and 6 retaining minute fragments), no deciduomata in non-engrafted controls (3 completely hypophysectomized and 6 retaining minute fragments).

In series II the test for progesterone secretion was vaginal mucification in the presence of an excess of estradiol benzoate (a total of 125 μg., in oil, given subcutaneously during the week preceding autopsy). Four rats were killed 33 days after transplantation, 1 at 50 days, 3 at 60 days and 1 at 90 days. All had mucified vaginas and each had a healthy set of large corpora lutea (ca. 2 mm.). These apparently constituted the original set; other parts of the ovaries were markedly atrophic.

In part the results confirm Desclin's (1950) conclusions: hypophyses isolated from the hypothalamus can nevertheless secrete luteotrophin. However, they further demonstrate that stimulation by estrogen, as Desclin employed it, is not essential; transplantation in itself favors luteotrophin secretion. It is suggested that during the usual short cycle of the rat the hypothalamus tends to inhibit this function, although incompletely, permitting only low-grade corpus luteum activity.

59. Correlated cytological and pharmacological observations on the release of histamine by mast cells. Don W. FAWCETT, Department of Anatomy, Harvard Medical School.

Experiments were designed to adduce more compelling evidence than is currently available that mast cells produce and release histamine. Solutions known to affect mast cells or known to release histamine were introduced in 20 ml. amounts into the peritoneal cavity of adult rats where they had access to a large population of mast cells in the serous membranes. At intervals, samples of the injected fluids were withdrawn and assayed for histamine on the atropinized guinea-pig ileum, while the associated morphology of the mast cells was studied in stained spreads of mesentery.

Injection of distilled water caused osmotic disruption of mast cells and release of small amounts of histamine (15–30 μg) into the peritoneal cavity. Injection of Tyrode solution containing 300 μg of the potent histamine liberator "Compound 48/80" resulted in release of many granules from mast cells and liberation of large amounts of histamine (100–190 μg). If the mast cells had been destroyed by intraperitoneal injection of distilled water several days earlier, then injection of "48/80" caused no release of histamine. These observations strongly support the thesis that mast cells are unusually rich in histamine which is released under experimental conditions which cause them to discharge granules.

15. The nervi tentorii and intracentral pain. William FEINDEL*, Department of Neurology and Neurosurgery, McGill University, and The Montreal Neurological Institute. (Introduced by F. L. McNaughton)

The origin and course of the nervi tentorii in man have been studied by osmic acid staining of autopsy specimens and by stimulation and subsequent novocaine block of their pain-conducting fibers during intracranial operations carried out under local anesthesia in conscious patients. They branch from the ophthalmic division of the trigeminal nerve to form a conspicuous nerve plexus which is distributed to the tentorium, falx cerebri, and occipital dura and along the venous sinuses enclosed by these leaves of dura. Some small bundles from the nervi tentorii also leave the dura to course along the large cerebral veins in the parieto-occipital region. There is an overlap of the territory of the nervi tentorii by fibers from the middle meningeal nerves and by the dural nerves from the posterior fossa. Many fibers in the nervi tentorii are larger in diameter than those usually considered to conduct pain impulses, and these may have other functions. The anatomical features of the nervi tentorii indicate their significance in relation to intracranial pain mechanisms.

146. Effect of gonadotrophic hormone on cross-strain grafting of ovaries between inbred strains of mice. Lloyd FERGUSON* and Arthur KIRSCHBAUM, Department of Anatomy, College of Medicine, University of Illinois.

Many adult normal tissues and organs of mice, e.g., ovary, lung, intestine, uterus, spleen, "take" when grafted into the subcutaneous tissue of the mouse ear if the donor tissue and recipient host are of the same inbred strain. If donor and recipient are of different strains, then the grafts may survive temporarily, but usually degenerate within 3 weeks.

The effect of gonadotrophic hormone on survival of ovarian grafts between strains was tested. The C57 BL and Strong A strains were used. Ovaries of 40-day-old mice were grafted into a subcutaneous pocket of the ear as follows: (1) C57 BL into gonadectomized and intact young adult C57 BL females and males, (2) C57 BL into castrated male Strong A's, (3) C57 BL into castrated male Strong A's which received 5 i.u. daily of pregnant mare serum.

Inter-strain ovarian grafts (from C57 BL into Strong A) all degenerated within 3 weeks unless the hosts were treated with pregnant mare serum. In these the ovarian grafts contained viable tissue for 5 weeks, when degeneration occurred although treatment was continued. Inter-strain grafts were hyper-stimulated by the gonadotrophic hormone as soon as vascularized.

Although intra-strain grafts "took" (6 week survival or greater) in either gonadectomized or intact mice, the ultimate fate of the graft was determined by the status of the host, that is, gonadectomized or intact, more grafts persisting indefinitely in gonadectomized mice.

Treatment of recipients with 400 r of x-rays, or 300 r plus 1 mg of cortisone daily, or cortisone alone did not improve inter-strain grafting. This procedure has resulted in temporary survival of human cancerous tissue in rats (Toolan, '51).

94. *The development of a strain of mice with a high incidence of adiposity.*[1] Frank H. J. FIGGE, Department of Anatomy, University of Maryland Medical School.

It was noted several years ago that there was a high incidence of adiposity in the progeny of black and white mice resulting from hybridizing a CBAN with a Strong A mouse. After the initial hybridization, the mice were inbred for several generations and selected for black and white coat color. While there are several strains of obese mice in existence, it appeared desirable to continue the inbreeding of these mice for the following reasons: The distribution of the fat and the age incidence of the adiposity appeared to parallel the anatomical distribution and age incidence of adiposity in the human subject. The mice in this strain which exhibited obese characteristics became overweight at the age of about 4 months. As the abnormally high rate of differentiation of fat cells continues, the mice of this strain reach an average weight of 1 1/2 times that of a normal mouse. The adipose members of this strain (50%) became 2 to 3 times the weight of a normal mouse. The weight curves of large numbers of these mice and mice of non-obese strains have been studied and compared. Data will be presented on other physical characteristics of these mice. The histology of endocrine organs and other structures will be discussed.

[1] Supported by a grant from the American Heart Association.

188. *Lethality of injected sodium cyanide toward neonatal mice.* Laurence R. FITZGERALD, Division of Anatomy, University of Tennessee.

When fresh, unbuffered solutions of sodium cyanide are injected subcutaneously into newborn and adult mice, marked differences between the two groups are noted. In newborn mice injected at 24–26°C, 30–70 minutes elapse before the animal is obviously dead, in contrast to the adult, which dies in less than 4 minutes.

If the ultimate death or survival of the animal is the only factor measured, the newborn is found to be more susceptible to this poison than the adult. The LD_{50} for newborn mice is about 2.3 mg./kg., whereas the LD_{50} for adult males and females is 5.0 and 3.4 respectively.

Since most of our information concerning the resistance of the newborn to anoxia and anoxic agents has come from studies in which the persistence of movements has been measured, the results presented here seem to call for a careful re-examination of the effects of anoxia.

189. *Apparent production of progesterone by human fetuses at or near term.*[1] Thomas R. FORBES, Department of Anatomy, Yale University.

One cc. blood samples were drawn from the umbilical arteries and veins of 15 infants born spontaneously or delivered by Caesarian section. All samples were obtained prior to placental separation and just before or after the cord was clamped. Each sample was citrated and promptly refrigerated. Plasma progesterone levels were determined by a bio-assay which is sensitive and relatively accurate; progesterone is the only known, naturally occurring compound which evokes a positive assay response.

Plasma from the umbilical artery of one baby contained < 0.4 microgram progesterone/cc., while the corresponding venous plasma contained 5.0 μg/cc. Progesterone levels were the same or nearly so in the umbilical arterial and corresponding venous plasmas of 5 babies, although progesterone concentrations ranged from less than detectable levels in one of these infants to 2.3 μg/cc. in another. Umbilical arterial plasmas from the remaining 9 babies contained 2–18 times as much progesterone (up to 4.6 μg/cc.) as the corresponding umbilical venous plasmas. When there is more progesterone in blood leaving than in blood returning to the fetus, it would appear that the fetus produces the hormone. The fetal source of the hormone has not been demonstrated; the adrenal cortex may be involved.

[1] Supported by a grant from the National Institutes of Health, Public Health Service.

134. Cleavage lines of the abdominal cavity. Joseph H. GARDNER*, Department of Anatomy, The Creighton University School of Medicine. (Introduced by R. Dale Smith)

Cleavage lines of the skin have been amply demonstrated in regard to their development and pattern formation. And it has been shown they represent a definite arrangement of collagenous connective tissue fibers; and are in reality lines of tension.

To date studies have been made only in regard to the skin. The present investigation was undertaken in order to determine (1) if cleavage lines existed in the body cavities and (2) if so, determine their pattern. Fetal specimens and adult cadavera were used. The peritoneum was pierced by a sharp conical instrument and the resulting linear wounds were painted black and photographed. Histologic studies were made from blocks of tissue containing the linear wounds that were sectioned in three planes.

A definite pattern was found to exist in the parietal and visceral peritoneum which reflected not only the influence of gravity but also the effects of internal forces in the viscera.

189. Energy rich phosphate and other chemical changes in the hearts of vitamin E deficient rabbits.[1,2] Arthur J. GATZ, Arthur G. MULDER*[3] and Blanche TIGERMAN*, Departments of Anatomy and Physiology, Stritch School of Medicine, Loyola University.

Twelve-hundred gram New Zealand giant rabbits were maintained on a vitamin E deficient diet. Littermate control rabbits received the same diet fortified with ephynal acetate (50 mg/kg). During the 4th week, the vitamin E deficient rabbits exhibited a marked weight loss, muscular weakness and finally paralysis (being unable to rise after being placed on their side). The electrocardiograms showed QRS and T wave changes. Previous reports, by one of us and others, have shown that some portions of the heart showed necrotic changes.

In this study the beating hearts were rapidly removed from the anesthetized rabbit, immediately frozen in a mixture of ether and dry ice and subsequently analyzed for inorganic phosphate, adenosine polyphosphate, creatine phosphate and glycogen. The inorganic phosphate, adenosine polyphosphate and glycogen

showed no significant change. The creatine phosphate showed a very marked reduction, averaging 6.1 mg per cent in the control rabbits and 0.9 mg per cent in the vitamin E deficient group ($P = 0.01$). The significance of these changes will be discussed.

[1] Supported in part by grant from The Chicago Heart Association to A.J.G.
[2] Aided by grant from The National Heart Institute to A.G.M.
[3] Deceased.

42. Analysis of sagittal plane forces for the seated subject.[1] George R. L. GAUGHRAN*, Department of Anatomy, University of Michigan. (Introduced by W. T. Dempster)

Conventional descriptions of muscle action deal with joint geometry and ignore effects of segment mass. This study shows how the body produces horizontal sagittal plane forces by utilization of body mass for subjects seated without foot or back rest. Side view photographs were taken simultaneous with records of vertical and horizontal force vectors at the moment of maximum push or pull. Estimated masses and centers of gravity of body parts marked on photographs permitted additional treatment of forces. Under the experimental conditions, up to 45 pounds of push or pull were found for a 140-pound man. By varying muscle tension over joint systems the subject can alter the position of the resultant vertical body component and produce increased or decreased lever arms. In *pushing*, the tendency was to extend the legs, shifting the center of gravity forward. Similarly, in pulling, the legs were flexed to move the center of gravity backward. Hip muscle tensions increased the effective torque by raising the head, thorax, and abdominopelvic masses so that the opposing force of the vertical couple was moved forward. The effectiveness of pulls and pushes was influenced both by position of mass and by shifting of fulcra.

[1] Supported by a contract with the Aero Medical Laboratory, Wright Air Development Center, Wright-Patterson A. F. Base, Ohio.

121. A quantitative study on the distribution of phosphorus^{32} in metamorphosing tadpoles (Rana sylvatica). Joseph F. GENNARO, Jr.*, Department of Biological Sciences, University of Pittsburgh, and Department of Anatomy, State University Medical Center of New York City. (Introduced by J. N. Dent)

Ten larvae of *Rana sylvatica* at each of 3 "premetamorphic" and 17 metamorphic stages of development were immersed individually for 24-hr periods in 25-ml volumes of spring water to which carrier free P^{32} (as $NaHPO_4$) had been added in the amount of 1 μc/ml. Immediately after removal from the radioactive solution, the gut, liver, pancreas, eyes, and hind limbs were dissected free and each, as well as the residual portion of carcass, was dried and weighed. The radioactivities of each of these structures was then measured and the relative concentration of P^{32}/mg dry weight calculated in each instance. When the values thus obtained were plotted against the stages of development, it was seen that, in general, wherever there was increase in mass, ossification, or secretion, there was a corresponding increase in the uptake of phosphorus in the tissues involved. Conversely, the atrophic changes of the gut were accompanied by decreases in phosphorus uptake. Changes in the P^{32} concentration of the liver apparently illustrate its role as a depot for the metabolites used in development.

183. Axon terminals in the human cerebrum and cerebellum.[1] William C. GIBSON*, Department of Neurological Research, University of British Columbia. (Introduced by S. M. Friedman)

Normal human cerebral and cerebellar tissue has been examined after staining with Rio-Hortega's Double Impregnation Method, using silver nitrate and silver carbonate. In the motor and visual areas, in the cingulate and hippocampal gyri, and in the sub-thalamus and cerebellum, *boutons terminaux* have been stained which closely resemble those occurring on the surface of the anterior horn cells of the spinal cord. The number of axonal endings stained varies considerably between different cortical areas. The ring-like endings occur on the dendrites as well as on the cell body of the neuron. Lantern slides will be shown illustrating their characteristics.

[1] Supported by a Federal Mental Health Grant.

17. Correlation of certain neurological dysfunctions with congenital anatomical abnormalities of the central nervous system in five cats. Lois A. GILLILAN and Isabel LOCKARD*, Department of Anatomy, Graduate School of Medicine, University of Pennsylvania, and Department of Anatomy, The Medical College of South Carolina.

It is possible to correlate certain functional disabilities with anatomical lesions whether they be traumatic or congenital. These correlations can be made on the basis of gross functional relationships to brain segments or in terms of specific function patterns to specific cell groups and fibers or both. In these animals a variety of "experimental lesions" was present but without the trauma of experimental surgery. The behavior of the cats was observed over a prolonged period of time and a detailed study was made of the gross and microscopical features of the brain. One cat with incapacitating motor disabilities was found to have widespread disorders involving motor centers including cerebral cortex, pyramidal tract, striatum and especially cerebellum. This cat was also very nearly blind and had a rudimentary lateral geniculate nucleus. Three cats showing extrapyramidal signs including a fine intention tremor and rigidity of the hind legs had abnormalities of the striatum. In addition, one of these animals was unable to jump even short distances from one level to another and land on his feet. This cat also exhibited a marked cellular derangement of the cerebellar cortex. A fifth cat was unsteady on his feet, had poor righting reflexes and had the high-stepping gait associated with cerebellar lesions. This animal had small cerebellar hemispheres and defective cerebellar nuclei.

92. Accumulation of periodic acid Schiff (P.A.S.) positive material during giant cell formation in vitro.[1] Milton GOLDSTEIN*, Department of Pathology, Western Reserve University. (Introduced by John W. Patterson)

In a previous paper a method was described for the rapid formation of multinucleated giant cells from monocytes of human blood. Small fragments of the buffy coat were explanted on the surface of perforated cellophane in D-5 Carrel flasks.

In the present study the periodic acid Schiff (P.A.S.) reaction was applied to these cultures after varying periods of incubation. The cultures were fixed in Carnoy's acetic alcohol for 20 minutes. The material was carried down to water and the P.A.S. reaction was performed. The P.A.S. reaction was negative in the monocytes in early cultures (one to 5 hours). As the cultures were incubated for longer periods of time there was an intense accumulation of P.A.S. positive material which was non-particulate in nature. The P.A.S. positive material which accumulated in the mononuclear cells and in the giant cells was not removed by the action of saliva, amylase, hyaluronidase, pepsin nor methanol/chloroform at 60° for 24 hours. Acetylation inhibited the P.A.S. reaction and treatment with potassium hydroxide reversed the effect of the acetylation. This substance appears to be a polysaccharide which has not as yet been identified by the investigator.

[1] Post doctoral fellow, National Cancer Institute, United States Public Health Service.

28. *The effect of denervation on the regeneration of the pectoral fins of fish.* Richard J. GOSS*, Department of Biology, Brown University. (Introduced by J. W. Wilson)

The fins of fish regenerate readily upon amputation, exhibiting the first macroscopic indications of growth after about two weeks, and regenerating rather rapidly thereafter. The nerves of the brachial plexus can be exposed by cutting away a section of bone from the lateral portion of the pectoral girdle, immediately behind the posterior margin of the opercelum. Denervation of the pectoral fin can then be accomplished without injuring the blood vessels supplying the fin. The left pectoral fins of catfish *(Ameiurus)* and goldfish *(Carassius)* were denervated in this manner, and simultaneous amputations of the distal halves of both fins were performed, the right fins serving as controls. Regeneration of the denervated fins was completely inhibited, and histological studies revealed that only wound healing had taken place and that blastema formation was not initiated. No regression was observed, as has been reported for denervated larval urodele limbs and catfish taste barbels. Furthermore, regeneration is arrested even when denervation is delayed up to 12 days after amputation. If denervation is not repeated, the nerve fibers grow back into the fin and regenerative processes start after 25 to 30 days. After 50 days the originally denervated fins regenerate nearly one-half as much as the control fins.

29. *Functional responses of the ovary of the immature mouse to equine pituitary gonadotrophin.* James A. GREEN*, Department of Anatomy, University of North Carolina. (Introduced by Charles W. Hooker)

The ovaries and uteri of 21-day-old mice of the A strain were examined at intervals of 12 hours for 72 hours after the first of a series of subcutaneous injections of a potent follicle stimulating gonadotrophin from horse pituitaries. The injections were given three times daily in individual amounts of 0.03, 0.42 and 3.75 R.U.

The ovaries of the animals receiving the lowest dose showed no increase in weight and little microscopic change. At the intermediate dose level the follicles

showed progressive stimulation, and luteinization began 60 hours after the first injection. At the highest dose level the follicles showed intense stimulation, and luteinization began 48 hours after the first injection. The condition of the endometrial stromal nuclei indicated the presence of progestin in all animals, with a decline accompanying follicular growth and a more intense return after luteinization. The weights of the uteri and the condition of the endometria suggested the initial presence of low levels of estrogen, followed by a slight increase in the animals given the lowest dose and by a conspicuous increase in the other animals. Upon luteinization, either no further increase in response to estrogen or a decline in intensity of response was indicated.

11. Electrical activity in hypothalamus and hippocampus of conscious rabbits. John D. GREEN, Department of Anatomy, University of California at Los Angeles and Veterans Administration Hospital, Long Beach.

Specially designed coaxial electrodes have been chronically implanted in the hippocampus and hypothalamus of rabbits and the electrical activity of these areas and of the cerebral cortex has been studied in unanesthetized animals, often unrestrained, for periods of up to 9 months. The electrical activity of the conscious rabbit hypothalamus is similar to that of the curarized guinea pig and, in the main, appears to be due to activity conducted to the hypothalamus from the hippocampus. Records have been obtained after coitus indicating possible changes in the anterior hypothalamus and changes in hippocampal activity during love-play. Castration appears to cause a moderate increase in hippocampal and hypothalamic activity, reversed after stilbestrol injections. The effects of change in body temperature, adrenalin and insulin injections have received preliminary attention without conclusive results. The hypothalamus shows the same "arousal" pattern reported previously in the hippocampus.

152. Sympathetic components of the vagus cardiac nerves of the dog. Stephen R. GREENBERG*,[1] Department of Anatomy, St. Louis University School of Medicine. (Introduced by Dr. Albert Kuntz)

Dogs were subjected to bilateral operative procedures. The superior cervical ganglia were removed in two animals, the intermediate or middle cervical ganglia in two, the inferior cervical ganglia in two and both the intermediate and the inferior cervical ganglia in two. Sufficient time was allowed following operation for complete degeneration of the interrupted fibers. The animals were then sacrificed, and the thoraces were removed in toto and fixed in 10% formalin. The cardiac plexus and the contributing rami were exposed by dissection in each specimen. Both the vagus cardiac and the cervical sympathetic cardiac nerves were removed individually and divided in two parts. One part was stained with osmic acid and subsequently sectioned. The other was sectioned and then stained by Holmes' silver impregnation technique.

All the cardiac rami of the vagus nerves convey both myelinated and unmyelinated fibers. The myelinated ones may be classified in three categories on the

basis of size: large, medium-sized and small. The unmyelinated fibers are chiefly sympathetic since they undergo degeneration following extirpation of the appropiate sympathetic trunk ganglia. They occur in circumscribed bundles within the vagus cardiac nerves.

[1] Life Insurance Medical Research Fund Fellow.

170. *The structure of intrasplenic ovaries in mice.*[1] Mary J. GUTHRIE, Detroit Institute of Cancer Research.

To determine the sequence of changes leading to the formation of intrasplenic ovarian tumors in mice, ovariectomy and implantation of one ovary into the spleen were performed in day-old mice. Intrasplenic ovaries were recovered at weekly intervals up to 14 weeks, later recoveries were fortnightly. Serial sections were studied.

Ovaries which remain entirely within the spleen are surrounded by a fibrous capsule and lose their germinal epithelium within a few weeks. Ovaries which slip partly or completely out of the spleen may retain part of their germinal epithelium as a single layer of cuboidal or squamous cells; in older transplants, it may be multilaminar in some sectors. Ingrowths from the germinal epithelium were not found.

The earliest observed differences between intrasplenic ovaries and those in situ occur during the 4th week when many of the multilaminar secondary follicles became luteinized and some tertiary ones became hemorrhagic. These changes are concurrent with an increase in number of interstitial cells which begin to be luteinized. Some of the small secondary follicles are not surrounded by thecal cells, and in regression their granulosa cells spread irregularly, forming cords and tubes.

Other follicles continue to begin to grow, lacking thecal cells. During the 12th week, pretumorous sites are established by unrestrained spreading of granulosa cells from nests of such regressing follicles.

[1] Aided by Institutional Grant from American Cancer Society.

128. *Effects of acetylcholine and of epinephrine on the embryonic rat heart.*[1] E. K. HALL, Department of Anatomy, University of Louisville School of Medicine.

The effects of acetylcholine (2.5 — 100 mg/kg — median: 20 mg/kg) and of epinephrine (1.25 — 50 mg/kg — median: 5 mg/kg) were tested on 148 embryonic rat hearts of the 10–14-day stages. The hearts were excised and control contraction rates determined in Krebs-Ringer at body temperature; averages ranged from 140.4/minute (10-day hearts) to 223.5 (14-day hearts). In Krebs-Ringer containing acetylcholine, 10-day hearts failed to arrest or to decelerate significantly; 11–14-day hearts however were significantly (18–58%) decelerated, and in 48% of the tests deceleration was preceded by a period (up to two minutes) of complete arrest.

Epinephrine effects were demonstrated by experimenting with the hearts in vitro, just after acetylcholine experimentation. One heart was transferred to

epinephrine-containing Ringer, the other (control) to Ringer. Significant (10–28%) acceleration was observed in some categories; the accelerated rates did not greatly exceed the control rates established before the acetylcholine experimentation. Evidently acetylcholine acts directly upon the myocardium of (pre-innervated) 11–13-day hearts and upon (innervated) 14-day hearts, but not upon 10-day hearts. The epinephrine effects, though variable, demonstrate the possibility of responses at stages before innervation.

[1] Supported by Grants-in-Aid from the American Cancer Society (Committee on Growth, National Research Council), the Kentucky State Medical Research Commission, and the National Heart Institute (Public Health Service).

102. Analysis of the action of propylthiouracil on the pituitary-thyroid axis.[1]

N. S. HALMI, Department of Anatomy, State University of Iowa.

Thyroid-serum iodide concentration (T/S) ratios and thyroid cell heights were measured in groups of Purina-fed hypophysectomized rats injected with thyrotrophin (TSH, .05–2.0 USP units daily for 5 days), propylthiouracil (PTU, 10 mg daily in suspension form for 10 days), or both substances (for 5 days).

The following results were obtained: (a) PTU caused a significant rise of the T/S ratio in the absence of TSH; (b) the action of TSH, as determined by thyroid cell height increments, was potentiated by simultaneous PTU administration; (c) TSH (1 USP unit daily) + PTU elevated the T/S ratio to 400 (mean cell height: 8.8 μ), whereas TSH alone (2 USP units daily) only raised the gradient to 140 (mean cell height: 9.0 μ). In uninjected controls the T/S ratio was 4.5 and the mean cell height 3.7 μ.

The finding under (c) cannot be attributed solely to potentiation of TSH by PTU. Rather, (a) and (c) together lend support to the thesis that, in addition to TSH, there exists a mechanism regulating thyroidal iodide uptake which is activated when the thyroid is depleted of iodine.

[1] Aided by grant A-423, USPHS.

136. Extracardiac anastomoses of the coronary arteries in the human newborn.[1]

Myron H. HALPERN*, Division of Anatomy, Hahnemann Medical College. (Introduced by A. W. Angulo)

The coronary arteries of the human newborn anastomose with vessels supplying large non-cardiac areas. Extracoronary structures may be vascularized by way of the terminal atrial branches of the coronary arteries.

Some of the distal atrial branches of the right coronary artery continue onto the ascending aorta, the right pulmonary vessels, and the superior vena cava as vasa vasorum. Branches of some of the atrial vessels from the left coronary artery provide vasa vasorum to the left pulmonary vessels. In addition, terminal branches of many of the atrial vessels of both coronary arteries supply an extensive retropericardial area. In this location, they freely anastomose with each other and with the bronchial and mediastinal arteries of aortic origin. Before becoming the vasa vasorum of the great vessels of the heart, some atrial arteries

send twigs to the pericardial sac. Anastomoses occur between these twigs and the pericardial branches of the pericardiacophrenic arteries.

Because of the practical significance of the collateral coronary circulation in man, further studies are in progress. The extent of the extracardiac coronary circulation in the human adult and common laboratory animals will be presented at a later date.

[1] Supported by grant H-1575 from U. S. Public Health Service.

22. Preliminary observations of the positions of the phagocytic von Kupffer cells in the living transilluminated livers of frogs and mammals.[1] Fann HARDING*, Department of Anatomy, The Medical College of South Carolina. (Introduced by Melvin H. Knisely)

Are the hepatic phagocytes a part of the sinusoid linings or are they "sternzellen" anchored across the sinusoids somewhat like spiders in tubes, partly obstructing blood flow?

The livers of healthy frogs, rats, mice, hamsters, and cats have been observed. Other species will be studied. A bloodless, sludgeless laparotomy exposes the liver which is transilluminated by quartz rod technique. In mammals, the lungs are continuously insufflated with oxygen by the Irwin and Macdonald (1953) technique which eliminates respiratory movements and permits detailed observations at from 50 to 660 diameters.

Observations: Blood flowed through the sinusoids unimpeded by any obstacles within the sinusoid lumina. The sinusoids are approximately cylinders. No stationary cells or any objects were seen suspended in or across the lumina of sinusoids.

To identify the position of the phagocyte, injections of black, colloidal mercuric sulfide were given. Black mercuric sulfide was seen within the material of the linings of sinusoids. No phagocyte was seen suspended in the lumina of any sinusoid.

In the transilluminated living livers of healthy, anesthetized animals where normal conditions of life are as closely preserved as possible, no "sternzellen" have been observed suspended within the lumen of any sinusoid. These studies indicate that the so-called "sternzellen" suspended across the sinusoid represent conditions imposed by death, such as collapse of sinusoids with subsequent fixation and staining.

[1] Supported by a grant from the Office of Naval Research (NR 114-065), Department of the Navy to the Medical College of South Carolina.

23. An experimental anatomical analysis of the topography and polarity of the striate-neocortex interrelationship in the primate. Pinckney J. HARMAN, Maleva TANKARD*, Christian HOVDE* and Fred A. METTLER, Department of Anatomy, New York University College of Medicine, and Departments of Anatomy and Neurology, Columbia University.

It is known that striatal shrinkage follows neocortical ablation; due, in part at least, to loss of cortical fibers of passage. It is not known whether such shrinkage has a corticotopic plan. Disagreement persists concerning cortico-striate and/or striatocortical projections.

We have studied the head of the caudate nucleus in cell and fiber preparations of 5 catarrhine monkeys sacrificed 3 months after unilateral decortication of (1) hemisphere (2) frontal lobe (3) lateral premotor surface (4) dorsolateral premotor surface (5) orbital surface. Data consist of planimetric measurements of cross-sectional areas and cell counts, from which are calculated volumes and cell numbers (corrected for atrophy).

Orbital removals result in fairly large reductions of rostral caudate volume with a *de facto* cell loss, moderate reduction of caudal caudate with no cell loss; ventrolateral is associated with moderate reduction and cell loss throughout; dorsolateral with moderate reduction and cell loss of caudal caudate only. Thus, ventral to dorsal in neocortex is represented as rostral to caudal in caudate. Atrophy of the Gudden type among the cells of the caudate supports the existence of a well defined pathway originating within the caudate nucleus and terminating in the neocortex.

16. Studies on the neural action of protoveratrine. Frank HARRISON and Andres GOTH*, Departments of Anatomy, Pharmacology and Pathology, Southwestern Medical School of The University of Texas.

Experiments with protoveratrine (4 microgm/kg) have been performed on cats and dogs under chloralose-urethane anesthesia in order to study the mechanism of the resulting hypotension. Bilateral section near the jugular foramen of the ninth and tenth nerves following protoveratrine injection permits the blood pressure, as recorded from the femoral artery, to return toward the control level. Insofar as these experiments have paralleled those recently reported by Moe and his co-authors, the latter work has been confirmed. In the few experiments so far completed on the effect of protoveratrine on the blood pressure rise to hypothalamic stimulation in the cat, there seems to be no marked change; and this in spite of the fact that protoveratrine potentiates the pressor effect of epinephrine. Since protoveratrine produces some additional fall in blood pressure when given after autonomic blockade (Etamon; or atropine and Regitine) it may have a direct peripheral action in addition to its apparent pressoreceptor sensitization. The possible mechanisms of this action are being investigated further. Protoveratrine does not alter to any marked degree the skin resistance decrease to hypothalamic stimulation in the cat.

86. Studies on the respiratory pattern of exudate leukocytes.[1] John D. HARTMAN, Department of Anatomy, Temple University.

This work has attempted to define the in vitro respiratory pattern of exudate leukocytes and to begin a study of the factors responsible for this pattern.

Guinea pig exudate leukocytes were suspended in a balanced salt solution. Oxygen uptake was measured by standard manometric methods, and respiration rates were expressed as mm^3O_2/million cells/hour. Cell counts were done on aliquots at half, one, and two hours. The respiratory pattern was demonstrated by plotting the changes in respiration rate with time.

If the respiration rate is measured at 30 minute intervals, there are no significant changes with time. If the respiration rate is measured at 10 minute

intervals, the respiratory pattern is quite variable for any one experiment. However, if the changes in respiration rate for several experiments are treated statistically, a characteristic curve is obtained. This curve is characterized by a rapid decrease in the respiration rate over the first 20 minutes and a gradual increase over the next 80 minutes. Serial cell counts indicate a definite destruction of the cells from shaking. Quantitatively the initial drop in respiration rate is not correlated with the decrease in number of cells. The period of maximum cell destruction corresponds to that phase of the respiratory pattern characterized by a slow increase in the respiration rate.

[1] Supported by U. S. Public Health Service grant E-525.

24. Direct visualization of mammalian glomeruli. Mary Ellen HARTMAN*, Department of Anatomy, Temple University. (Introduced by John Franklin Huber)

Using the quartz rod technique and a combination of oblique and transillumination it is possible to visualize active glomeruli in the kidney of the 4 to 5 gm mouse. At this age the primitive zone of cortical material lying immediately under the capsule has almost completely disappeared; and, since the overgrowth of tubules has not yet begun, many glomeruli lie at or near the surface of the kidney. The glomeruli were observed in approximately 50% of the animals attempted. The failures are attributable to the hazards of anesthesia in this size animal, and to operative trauma. The glomeruli range in size from 29 to 53 μ, and an individual glomerulus has been observed for as long as one and a half hours.

Prior to the onset of intravascular agglutination capillary intermittence occurs but glomerular intermittence usually does not. Only rarely has glomerular intermittence preceded the onset of intravascular agglutination. Following the appearance of intravascular agglutination total glomerular intermittence may occur.

7. Segmental anatomy of the liver with special reference to partial hepatectomy. John E. HEALEY, Jr., Jefferson Medical College.

Although radical operations upon the liver have been performed from as early as 1886, the surgical technic of such resections has changed very little. This has been mainly due to the fact that surgeons have been unaware of the intrahepatic course and distribution of the larger vascular and biliary channels and that, therefore, incisions through the hepatic parenchyme have been rather blind and crude in performance.

From an examination of the liver casts in which the bile ducts, hepatic artery, and portal vein were injected with a vinyl acetate solution certain fissures were observed which divided the liver into its lobes and segments. These three systems were segmentally arranged and closely followed one another in their intrahepatic course.

The hepatic veins were not segmentally arranged, however; the main trunks of these veins were found in the intersegmental planes and drained adjacent segments. The aforementioned fissures could not be seen in casts in which the hepatic veins were injected.

With the establishment of a prevailing pattern of intrahepatic gross anatomy, radical liver surgery may be performed on a more sound surgical-anatomical basis; i.e., by means of the individual ligation technic, and thereby increase the operability and lower the mortality of such procedures.

107. *The ground substance of the developing central nervous system.*[1] Arthur HESS, Department of Anatomy, Washington University School of Medicine.

In the gray matter of the central nervous system, a homogeneous substance occupies the spaces between cells, dendrites, axon terminations and neuroglia fibrils. On the basis of histochemical reactions, this substance has been classed tentatively as a neutral mucopolysaccharide and has been regarded as an intercellular ground substance of adult central nervous system (Hess, '53). Brains of 32 guinea pig fetuses, varying in gestation age from 39 to 60 days, were subjected to the periodic acid-Schiff (PAS) procedure. Occurrence of the PAS-positive ground substance in the cerebral cortex was noted and a rating assigned to the amount and intensity of staining of this substance. The ground substance makes its appearance at 45 days in fetal guinea pigs. It thereafter increases in amount and in intensity of staining until by 50 days fetal age, it is similar to that of the adult. The source of this ground substance is discussed. Neurons and vascular feet of astrocytes are suggested as elements responsible for elaboration of this substance. A pathological role for the ground substance in cerebral edema and swelling is suggested. It is also suggested that the ground substance provides the colloidal substrate and is the material responsible for orientation of developing fibers of the central nervous system to their destination or synapse.

[1] Supported by a grant from the Public Health Service (B-341).

1. *Monozygotic twin human embryos with an estimated ovulation age of 17 days.* Chester H. HEUSER, Department of Microscopic Anatomy, Medical College of Georgia.

This specimen was obtained from Dr. Edgar R. Pund, Professor of Pathology, Medical College of Georgia, who discovered the implanted ovum in a uterus removed surgically because of cervical disease. The external measurements of the chorion were approximately 6 × 10 × 14 mm. By dissection of the chorion it was found to contain presomite twin embryos which appeared normal in form each with its own amnion and spherical yolk sac — and attached about 4.4 mm. apart. The sagittal sections of the embryos and plastic sheet reconstructions permit ready comparison. The lengths of the germ disks, measured with a flexible scale, are approximately 0.9 and 0.66 mm. The structural details are similar in the two embryos. In each the primitive node, situated in the center of the disk, the notochordal process which reveals the first evidence of canal formation, primitive streak and allantoic duct are well established. Angioblastic tissue is normal for this stage of development (Horizon VIII). The specimen is believed to be the earliest known example of twin formation in man and it presumably emerged from a morula in which two groups of formative cells were demarcated simultaneously quite apart from each other.

122. Development of cardiac myofibrils as seen with the electron microscope. Richard G. HIBBS*, Department of Anatomy, University of Minnesota. (Introduced by J. Francis Hartmann)

Development of cardiac muscle in the chick embryo was followed from the 2nd day of incubation, when no indication of myofibrils was seen, through the 6th day, at which time many myofibrils of adult type were present.

Sections of ventricular tissue, fixed in buffered OsO_4, were studied with the electron microscope. In early stages, the muscle fibers are spindle-shaped, and resemble smooth muscle cells. The cytoplasm is homogeneous in appearance, and contains many longitudinally arranged submicroscopic filaments. Numerous mitochondria are present near the nuclei. The first appearance of myofibrils occurs during the 3rd day, when they first appear as parallel groupings of the isodiametric longitudinal filaments. Some of these groups exhibit dark bands that correspond to the Z bands of later stages. By the 4th day, well-formed myofibrils with complete banding were seen in the cytoplasm. The A bands show great variation in appearance from one myofibril to another, some being very dense, others very light, indicating that the accumulation of "A substance" is gradual. The widest range of developmental stages among individual myofibrils was found between the 4th and 6th days of incubation. In areas containing differentiating myofibrils, numerous mitochondria (identifiable by the mitochondrial crests) were observed, many of them being arranged along the edges of the myofibrils.

195. Temperature regulation in the opossum. A. C. HIGGINBOTHAM*, Department of Physiology, Florida State University. (Introduced by Elsie Taber)

Most of the studies on temperature regulation have been on mammals high in the evolutionary scale. Little has been done on primitive forms.

When ambient air temperature was increased about 1° every fifteen minutes over a range of 30°–40°C., the rectal temperature of young opossums gradually rose from about 34° to about 39.5°C. That of mature animals increased at about the same rate but rose only to about 38.5°, indicating a more effective mechanism in the adults. As their rectal temperatures approached 38°, both young and adult animals became restless and spread saliva over their legs and tails by licking themselves. Panting started at about the same time. Sweating could not be detected on any part of the body. Regional surface temperatures gave no indication of a differential increase in peripheral circulation.

Anesthetized adults similarly heated began panting rather suddenly at a rectal temperature of 38°. No licking occurred. Rectal temperature reached a higher level than in conscious animals.

These experiments indicate that the adult conscious animals use the evaporation of saliva spread upon their fur and tails as an indispensable part of their heat dissipating mechanisms.

73. *Some histochemical studies of epiphyseal cartilage of rachitic rats, with relation to calcification in vitro.* Albert HIRSCHMAN*, Department of Anatomy, State University of New York, College of Medicine at New York City. (Introduced by Jack Gross)

White rats were maintained on Rachitogenic Diet no. 2, U.S.P. (enriched with 2% brewers yeast) for three weeks after weaning. Longitudinal sections of tibia were cut (0.2–0.4 mm. thick) and incubated in a basal salt solution or a calcifying solution for 5–6 hours. The sections were fixed in 4% basic lead acetate in 10% formalin, Rossman's fixative, and 10% formalin, and sectioned at 10 micra.

The Von Kossa stain showed a blackening of the matrix in the region of the most recently hypertrophied chondrocytes, in only those sections incubated in the calcifying solution.

The PAS reaction was strong in the matrix and weak in the chondrocytes. In the matrix, the reaction localized mostly on the fibrils and on the capsules of the chondrocytes. It appeared to be more intense in the region of *in vitro* calcification. The control sections showed a very weak staining of cells and matrix.

Metachromasia was strongest in the region of proliferating cartilage, being mostly localized in the capsule. It did not appear to have any definite relationship to *in vitro* calcification.

Sudan black B stained deeply in the chondrocytes, but was absent in the matrix. Oil red O and Sudan IV showed a similar localization to Sudan black, but the staining was much weaker. Lipid distribution did not change during *in vitro* calcification.

78. *Influence of dietary liver upon initiation of spermatogenesis in immature rats.*[1] Eugene H. HORN*, Bureau of Biological Research, Rutgers University; Department of Anatomy, Albany Medical College. (Introduced by Richard A. Miller)

A synthetic protein-free diet containing 5% dessicated liver powder was fed to immature male rats for 30 days during which time maturation of the reproductive system was thereby prevented. Transfer of these animals to an 18% casein diet for 20 days resulted in a marked, although incomplete, testis weight recovery which was slightly greater than that found in controls on an 18% casein diet, initially fed a diet free of both protein and liver. However, mature spermatozoa occurred in 75% of liver-fed animals as opposed to only 12% in the controls. Additions of 0.2% thyroid to the protein-free diet in the presence of liver permitted the appearance of mature spermatozoa in 25% of the animals after refeeding 20 days on an adequate diet. Previous investigation (Howell; Leathem, 1953) revealed a 77% mortality in immature rats fed a protein-free diet with 0.2% thyroid, but without liver supplements. Accessory organs failed to recover in all instances. Although testis weight recovery was

only slightly influenced by the liver powder, the maturation of spermatozoa was decidedly augmented, even in the hyperthyroid animals.

The increased testicular lipid and decreased RNA and DNA, histochemically determined, in all experimental groups was little affected by liver supplements.

[1] Supported by the Protein Metabolism Fund, Rutgers University.

38. Studies on the nature of the boundaries between broncho-pulmonary segments. John Franklin HUBER, Department of Anatomy, Temple University School of Medicine.

At the boundary between adjacent segments the alveoli of one segment are separated from the alveoli of the other segment by a small amount of fibroelastic connective tissue. The tributaries of the pulmonary veins which drain blood from the segments are in this connective tissue, the corresponding arteries being centrally placed in the segments in relation with the bronchi. Studies designed for the purpose of learning more about the possibilities of passage between segments were carried out as follows: A water suspension of pigment particles 3–5 μ in diameter was allowed to run slowly through a canula tied into a segmental bronchus from a reservoir about 15 inches above the specimen. About 4000 cc of the suspension ran in during 1½ to 2 hours. The segment into which the canula was tied became distended and deeply colored. In a significant number of instances the adjacent segments received no apparent pigment granules although these segments became distended with water. All of the water except that filling the lobe of the lung in which the colored segment was located, leaked out through the pulmonary arteries and veins and the bronchi of the adjacent segments. A greater percentage of the water came out through the arteries and veins than through the bronchi.

135. Non-specific esterase in the embryonic development of the frog, Rana pipiens.[1] Robert L. HUNTER*, Department of Anatomy, University of Michigan. (Introduced by Bradley M. Patten)

A modification of the method of Huggins and Lapides (1947) for the quantitative estimation of esterases was used to investigate the amount of non-specific esterase in the egg, embryo and young larva of the frog, *Rana pipiens*. The substrate used was p-nitrophenol propionate. The total amount of non-specific esterase per embryo was found to decrease from the time of fertilization to the tailbud stage. Transverse segments of the tailbud stage were found to contain non-specific esterase approximately in proportion to their size whereas a median frontal section divided the embryo into a dorsal part containing 29% of the enzyme and the ventral part containing 71% of the enzyme. This concentration of enzyme in the ventral moiety was shown to be due to enzyme in the yolk-laden gut.

Larvae of Shumway stage 16 and older hydrolyzed the substrate while still intact. This capacity was reduced one third following cardiectomy although the

amount of enzyme in the larva was not substantially reduced. Removal of the larva from the medium stopped the substrate hydrolysis. This suggests that the hydrolysis of the substrate by the intact larva was not due to the secretion of enzyme into the medium.

[1] Supported in part by a Predoctoral Research Fellowship from the Atomic Energy Commission.

131. Skin transplantation in the rat. Myron S. JACOBS*, Graduate School of Arts and Science, New York University. (Introduced by Earl O. Butcher)

Skin from offspring of predominantly black hooded rats was used as donor tissue. The donors ranged in age from 12 day fetuses to 21-day-old postpartal rats. Litters of albino rats ranging from 17 to 48 days in age were employed as hosts. Subcutaneous implantation of donor skin resulted in the formation of palpable cysts within a week. Cysts of fetal origin remained large and movable for many months, while those postpartal in nature appeared to attain maximum size within 2 weeks and then they gradually decreased.

Following a 2 to 4 week implantation period, the majority of cysts were exteriorized. Exteriorization consisted of removing a small piece of overlying host skin, incising and flapping open the cyst, and suturing the peripheral border of the opened cyst wall to the cut host skin edge. Many cysts contained black hair as well as fluid and sebum.

To prevent infection and mechanical destruction of the grafts, terramycin ointment was applied topically and each animal was placed in a restraining bandage. Periodic examination revealed that most of the postpartal grafts dried up and sloughed. In several fetal grafts apparent permanent attachment was effected. Pigmented hair continued to grow from the graft and appeared to parallel the alternating stages of the host hair cycle. At present these successful homografts have been followed up to 6 months.

127. A histochemical study of the glycogen in the nervous system of the developing salamander, Amblystoma maculatum.[1] Ivan D. JANOSKY*, Department of Anatomy, University of Kansas. (Introduced by Paul G. Roofe)

A histochemical study of glycogen in the nervous system of *Amblystoma maculatum* has been made. This study was concerned with the embryonic stages of development described by Coghill. A total of 126 animals was prepared and studied using the periodic acid-Schiff method.

Glycogen was found in varying degrees in all parts of the nervous system except in the cell nuclei which appear to be completely negative. The axons connecting ganglia to the brain are, in most cases, more glycogen positive outside than inside the meninges, the line of demarcation being sharp.

In general, there is an overall increase in glycogen activity from the non-motile stage to the early flexure stage followed by a decrease from then until the early swimming stage. The notable exceptions to this are the retina (rods and cones),

the choroid plexus, the epiphysis and Mauthner's neuron. In these areas, glycogen activity is higher in later stages than in the earlier ones.

The production and use of glycogen is one of the characteristics of nervous tissue. It is synthesized and stored in the cells of the central nervous system during proliferation and is rapidly metabolized in the active process of differentiation.

[1] Supported by a grant from the National Institutes of Health, U. S. Public Health Service, B 113 (C2).

72. *Rate of individual Haversian system formation.*[1] Webster S. S. JEE* and James S. ARNOLD*, Radiobiology Laboratory and Department of Anatomy, University of Utah, College of Medicine. (Introduced by Edward I. Hashimoto)

As a part of a series of experiments on bone growth and calcium metabolism, the rate of formation of individual secondary Haversian systems was investigated radioautographically using Ca^{45} in the tibiae of growing rabbits. Studies were made on animals sacrificed at 1, 7 and 21 days following Ca^{45} administration (Arnold and Jee, '53). Bone which was actively depositing at the time of Ca^{45} administration was detected by its ability to concentrate Ca^{45}. All secondary Haversian systems which concentrated Ca^{45} were classified as to the stage of formation at both time of injection and time of sacrifice. The data indicates that the rate of bone apposition is greatest during the first half of the process of Haversian system formation (filling of a resorption cavity). This is demonstrated by two types of data: (1) the frequency distribution of various stages of formation and (2) direct measurement of bone deposition in individual Haversian systems after 7 and 21 days. At the time of Ca^{45} administration 60–70% of forming Haversian systems of all animals were more than half completed. Growth arrest was frequently observed in all stages of formation. Of the Haversian systems which were just beginning formation at the time of Ca^{45} administration, 20–30% were completed after 21 days.

[1] Supported by AEC Contract AT(11-1)-119 and the U. S. Public Health Service.

73. *Anatomical distribution of strychnine paroxysms in the brain stem.* Beaumont JOHNSON*, Department of Anatomy, Northwestern University Medical School.[1] (Introduced by Ray S. Snider)

If small doses of strychnine are administered intravenously to the unanesthetized cat, prolonged electrical paroxysms are observed in the electrocorticogram of the cerebellum but not of the cerebrum. This activity approximates 200 microvolts at 18 per second.

In 12 curarized cats such paroxysms were incited and their continuation into the brain stem was mapped through the use of bipolar concentric electrodes and recorded by an ink-writing oscillograph. The multiple electrodes were stereotaxically oriented as a gang and their positions verified histologically.

The core of this activity conspicuously resided in the brachium conjunctivum, red nucleus, fields of Forel; caudally it scattered into the superior and inferior colliculi, midline bulbar reticular formation, inferior olive and inferior cerebel-

lar peduncle. The ventralis lateralis, a known thalamic termination of the superior cerebellar peduncle, was excluded from the paroxysm as was the central thalamic nuclear group. Certain systems did not actively particpiate as a whole, namely, pontine, vestibular and pyramidal.

The physiological aspects of this phenomenon are being studied.

[1] Aided by contract with the Office of Naval Research.

57. *Experimental study of spino-reticular connections in the cat.* Fred H. JOHNSON*, Division of Anatomy, Hahnemann Medical College. (Introduced by R. C. Truex)

Cajal described a system of collaterals from the spino-thalamic and ventral spino-cerebellar tracts which terminated in the reticular formation. This and other collateral systems of ascending tracts were studied by placing lesions in the spinal cord. Cats were killed on post-operative days, 3, 4 and 7. The brain stems were prepared by Nauta's method.

Spino-reticular connections to lateral reticular nucleus described by Brodal, and spino-olivary connections (Brodal, Walberg, and Blackstad) using Glee's method are confirmed. The observations based on Marchi preparations showing that the spino-reticular system is extensive and extends medially (Morin, Schwartz, and O'Leary) are corroborated.

Distribution of degenerated axons in the nuclei of the reticular formation were as follows (Olszewski's terminology): subnucleus reticularis dorsalis and ventralis medullae oblongatae, reticularis gigantocellularis and its pars alpha, lateralis oralis medullae oblongatae and subnucleus tractus spinalis trigemini oralis, reticularis pontis caudalis and its pars alpha, reticularis pontis oralis. Nucleus reticularis parvocellularis is almost devoid of degenerating axons, as is the nucleus reticularis superioris pars lateralis of von Bechterew.

Other systems that degenerated were: spino-vestibular tract of Collier and Buzzard terminating in Deiter's nucleus; spino-aqueductal of Le Gros Clark terminating in aqueductal gray at rostral level of inferior colliculus; axons terminating in VII nucleus; spino-pontine system terminating in lateral dorsal nucleus. No degenerating fibers issue from cortico-spinal tract. All the above collateral systems are principally homolateral.

26. *Results of kidney tissue auto- and homo-transplants in single and parabiotic albino rats.* Benjamin B. KAMRIN*, Department of Anatomy, State University of New York, College of Medicine at New York City, and Robert P. KAMRIN*, Department of Zoology, Cornell University. (Introduced by J. P. Parnell)

Autogenous transplantation of slices of kidney tissues has been reported in the literature as being wholly unsuccessful. Schnapauff (Beitr. z. Path. Anat., 1928) concludes that such transplants to the kidney or elsewhere become areas of infarction and connective tissue replacement within a period of three weeks. Homo-transplantation of slices of kidney tissue results in even earlier destruction of the transplant.

In the present investigation, the fate of slices of kidney tissues, severed and reimplanted on the same kidney in 20 albino rats, and the transplantation of homogenous slices of kidney tissue between both parabionts of 12 successful parabiotic pairs, was explored. In all the operated animals, the remaining healthy kidneys were removed within one month. The animals were sacrificed 6 to 12 weeks after the initial implantation or transplantation.

Results of these procedures demonstrate that in 50 per cent of the single animals, such auto-transplants remain histologically viable at the end of the experiment. The homo-transplants were viable in more than 50 per cent of the animals in successful parabiosis and presented the same histological picture as the auto-transplants. Injection of thorotrast into the renal artery and ureter demonstrated union of the transplant with the original kidney.

125. Incorporation of radioactive glycine into proteins of developing oocytes of Rana pipiens.[1] Norman E. KEMP, Department of Zoology, University of Michigan.

Adult female frogs were injected with solutions containing 10 or 20 microcuries of glycine-2-C-14. Animals were sacrificed at various intervals up to 5 days after injection. Samples of selected organs (heart, lung, liver, stomach, intestine, ovary, kidney, spleen and brain) were fixed for sectioning. Other samples of the same organs (brain omitted) and of blood were homogenized in 10% trichloracetic acid to precipitate proteins. Geiger counts of sections revealed the greatest radioactivity in liver, stomach and ovary of most specimens. Intestine, kidney and spleen usually had intermediate activity; heart, lung and brain, the lowest activity. Autoradiograms confirmed these findings and revealed localization of active protein synthesis. In the ovary uptake of radioactive glycine was demonstrable chiefly in the outer cytoplasm where yolk was being deposited. Further evidence that uptake of glycine was correlated with synthesis of yolk came from Geiger counts of isolated proteins. Samples of protein from ovaries containing oocytes in stages after the initiation of yolk deposition had higher specific activities than samples from ovaries in pre-yolk stages.

[1] Aided by a grant from the Michigan Memorial-Phoenix Project.

156. The course of visceral branches of sacral nerves to the rectum and distal colon in human fetuses.[1] Donald L. KIMMEL and Lowrain E. MC CREA*, Departments of Anatomy and Urology, Temple University School of Medicine.

Visceral branches of sacral nerves reach the rectum and distal colon by 3 routes: (a) by direct branches to the lower rectum, (b) by direct branches to the posterior rectal wall, and (c) by indirect branches via the pelvic plexus.

The direct branches to the lower rectum are branches of the fourth sacral nerve. They course caudad between the levator ani and rectum to terminate on the walls of the ampulla and anal canal.

The direct branches to the posterior rectal wall arise as 6–8 independent branches from the third and fourth sacral nerves. They course medially to join the nerve plexus on the posterior wall of the rectum. The upper branches of this group join the plexus surrounding the superior hemorrhoidal artery and follow its anastomotic branches to the sigmoid colon.

The indirect branches via the pelvic plexus arise from the second, third and fourth sacral nerves. Within the pelvic plexus nerve fibers of sacral origin can be followed dorsally to enter the rectal wall. Of the others, many turn caudad within or lateral to the pelvic plexus to supply the anterior pelvic viscera. Several other bundles turn rostrad to supply the descending and sigmoid colon. These can be followed only a short distance before they are lost in the superior hemorrhoidal or the hypogastric plexuses.

[1] Aided by a grant from Hoffman LaRoche, Inc.

91. *Heteroplastic mitosis in leukoblastic cells responsible for the formation of ring nuclei.* Riojun KINOSITA*, Susumu OHNO* and Jeremy P. WARD*, Departments of Infectious Diseases and Pathology, School of Medicine, University of California at Los Angeles and Medical Research Institute, City of Hope Medical Center. (Introduced by Horace W. Magoun)

A high percentage of atypical mitotic figures, unlike those of typical homoplastic division, were observed in cells of normal rat bone marrow. One type of atypical figure was a ring form. Ring anaphase figures were frequently observed. The ring form remained through telophase and eventually showed commencement of nuclear reconstruction. The daughter cells at the final stage of division presented the appearance of ring myelocytes or when accompanied by cytoplasmic maturation, the appearance of ring leukocytes. Ring and segmented ring metaphase figures were occasionally observed. Some of these figures showed chromosomal resolution and even nucleolar formation, indicating reversion of mitosis directly to the resting nuclei of the corresponding forms. The reversion from metaphase being accompanied by cytoplasmic maturation, resulted in cells showing an appearance transitional to ring and segmented ring leukocytes. As a result of this study it is believed that ring and segmented ring nuclei of myelocytes and leukocytes, which are difficult to interpret in terms of the prevailing concepts of serial maturation, are actually the result of heteroplastic mitosis of the myelocytes.

158. *Regenerative ability of the preganglionic fibers of the cervical sympathetic trunk of the cat.*[1] Homer D. KIRGIS, Departments of Anatomy and Surgery, School of Medicine, Tulane University and Section on Neurosurgery, Ochsner Clinic.

The ability of the preganglionic fibers of the cervical sympathetic trunk of the cat to regenerate long distances and through tissue barriers has been investigated. The extensive recovery of function of sympathectomized smooth muscle of the cephalic region of the cat following excision of the cervicothoracic gan-

glion has been reported. That such recovery of function might be due to the re-establishment of sympathetic connections in the ganglia of the inferior portion of the cervical sympathetic trunk instead of by regeneration of the preganglionic fibers throughout the length of the cervical sympathetic trunk following cervicothoracic ganglionectomy has been excluded in the present experiment by removal of the middle cervical sympathetic ganglion. The sympathectomized muscles demonstrated recovery of approximately normal function in the six experimental animals used. The ability of the preganglionic fibers to regenerate through or about tissue barriers was investigated in three cats by removing a segment of the sympathetic trunk 1 cm in length immediately inferior to the superior cervical ganglion. A ligature was placed tightly about the lower pole of the ganglion and the latter turned and firmly anchored with its longitudinal axis at a right angle to its normal position. The carotid sheath and its contents were fixed by sutures between the upper end of the severed trunk and the ganglion. Recovery of function of the sympathectomized muscles occurred in each animal.

[1] This investigation was supported by a research grant [PHS B-496 (C5)] from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service.

192. *Specifically localized, fibroma-like tumors induced in Syrian hamsters by treatment with androgen and estrogen administered simultaneously.*[1] Hadley KIRKMAN, Lynn ROBBINS* and Masako A. BABA*, Department of Anatomy, Stanford University School of Medicine.

Benign tumors develop in the tela subcutanea under the pigmented costovertebral spots of Syrian hamsters implanted subcutaneously with pellets of diethylstilbestrol and testosterone propionate. When the spots are excised prior to treatment the tumors fail to develop. When the spots are transplanted to the abdomen the tumors develop under them there, but not at the original site. The tumors may be induced equally well in either sex, intact or gonadectomized. Following removal of the pellets the tumors stop growing, but show no regression. The shortest period of treatment followed by grossly visible tumors is 59 days; ordinarily tumors are not expected within less than 150 or 200 days of treatment. Progesterone does not prevent tumor induction. After the tumor has appeared the spot may be removed without interfering with tumor growth, or a tiny fragment may be transplanted subcutaneously, or to the cheek pouch, where it grows quite independently of pigment, but only in the presence of estrogen and androgen. Hypophysectomy does not stop growth of the tumor or interfere with its successful transplantation. Subcutaneous transplants metastasize regularly to the lungs. Somewhat similar, multiple tumors may be induced by testosterone propionate alone, but only after a relatively long latent period; these androgen-induced tumors have never exceeded a millimeter or two in diameter however, even after 900 days of treatment.

[1] Supported by a grant from the American Cancer Society.

20. Preliminary observations of the catch-trap architecture of pulmonary artery tips in health and their responses following distant somatic burns.[1] William H. KNISELY* and Melvin H. KNISELY, Department of Anatomy, The Medical College of South Carolina.

Living transilluminated lungs of anesthetized young and old cats and dogs, and of mature rabbits have been observed. The lungs were supplied with oxygen by the Irwin and Macdonald (1953) technique, which eliminated respiratory movements and permitted detailed observations at from 50 to 950 diameters. A bloodless, sludgeless thoracotomy exposed the edges of lung lobes.

Living pulmonary artery tips have a special architecture. They are wide and have rounded, blunt tips. For long distances the walls of the sides and ends of the blunt tips consist largely of the rims of the afferent orifices of pre-capillary and true capillary-sized vessels.

At the instant of burning an animal across the back and rump, many visible terminal pulmonary arteries constricted completely shut. Shortly, most of them reopened.

Within 30 seconds to one minute masses of agglutinated blood cells, some large, some small, came down into pulmonary artery tips. The blunt, porous-walled pulmonary artery tips trapped the largest masses immediately. Blood continued to flow down the arteries around and over the trapped masses.

Trapped masses slowly broke up, perhaps affected by chemicals from passing blood or adjacent tissues.

Thus, pulmonary artery tips have a "catch-trap" architecture which catches and holds some sludge masses until they disintegrate.

[1] Supported by a grant from the Office of Naval Research (NR114-065), Department of the Navy to the Medical College of South Carolina.

160. Studies of basic dye-nucleic acid interaction.[1] Harold KOENIG, Ruth KOENIG*, Jerome EISENBERG* and Daniel SCHILDKRAUT*, Department of Anatomy, Chicago Medical School.

Thionin in aqueous buffer at low pH (3.5) stains nucleic acid-bearing structures selectively in formalin-fixed sections of nervous tissue, no differentiation being required. Microspectrophotometry of stained nerve cell cytoplasm reveals interesting alterations in the absorption spectrum of the bound dye. The amount of dye bound by cytoplasmic ribonucleic acid as measured by the D_{600mu} reaches a maximum value after 20 minutes of staining and remains unaltered even after 18 hours of staining. The staining of nerve cell cytoplasm follows Lambert's law, i.e., staining intensity as measured by D_{600mu} is a linear function of section thickness. These data suggest that nucleic acid and thionin under these conditions of staining combine in a stoichiometric manner and that such staining can be utilized for quantitation of nucleic acid in tissue sections by microspectrophotometry. The question is being investigated biochemically with fixed homogenized brain which is stained with thionin under standard conditions that duplicate the staining of tissue sections. After washing out of excess dye, the nucleic acid

and dye are extracted with perchloric acid and the phosphorus, sugar and nitrogenous bases of the nucleic acid determined along with dye. Preliminary data indicate that thionin combines with nucleic acid in the proportion of one mole of the dye to two moles of nucleic acid phosphorus.

[1] Supported in part by a grant-in-aid from the U. S. Public Health Service.

121. The morphology of the contractile apparatus of papillary muscle. S. I. KORNHAUSER and George R. SCHRODT*, Department of Anatomy, University of Louisville School of Medicine.

Material was sheep hearts, Helly fixed, stained with silver for reticular fibers and Crossmon's acid-fuchsin fast-green for tissue detail. The Z-lines not only extend across the muscle fibers through the sarcoplasm, but beyond to the interfiber reticular network, where they can be clearly seen connecting with fairly coarse longitudinal reticular fibers and pericapillary reticular networks. The connections with the fairly coarse longitudinal fibers produce a zig-zag appearance resembling a closed zipper. The longitudinal reticular fibers are connected with a fine transverse set lying a sarcomere or less apart. This forms a close network about every cardiac muscle fiber and capillary, all intimately united with the Z-lines at the periphery of every muscle fiber. The longitudinal reticular fibers converge and unite into strong bands as they reach the apex of the muscle. Lying between the terminal cardiac fibers these coarse silver stained strands unite with the green collagenous fibers of the chordae tendineae. In the area of union numerous fibrocyte nuclei and capillaries are seen. The distal ends of the terminal cardiac muscle fibers are only tenuously connected by means of a few green staining fibers. Thus the traction of the muscle upon the chordae is transmitted from the sides of each muscle fiber through the Z-lines to the reticular network, then to the strong longitudinal reticular strands and finally to the chordae.

65. Connections of the superior parietal gyrus (area 5 or PE) in the monkey. Wendell J. S. KRIEG, Department of Anatomy, Northwestern University Medical School.

Dorsal area 5 sends a strong albal stalk ventrally and forward, becoming narrow at bifurcation above caudate. The surface is represented topically as sagittal bands. The projectional branch drops ventrally into lateral end of peduncle and ends among lateral and dorsal pontile nuclei, except for a few fibers which continue through bulbar pyramid. None enter tegmentum. The callosal branch is weak and finefibered but widely distributed, though not to homotopic site. Corticothalamic fibers are very weak, but pass to ventralis posterior. Associational connections are rare backwards, and lacking for area 7 laterally, or below cingulate sulcus, or temporal or prefrontal lobes; numerous to area 5 forwards, and area 2; but lacking for postcentral dimple between these. A scattering run down postcentral alba and end along area 1, but not in 3. A great number congregate in paracentral alba, branch profusely, terminating massively in 4 f, g (gyyral) dorsally, sparsely in 4 h (sulcal), and 4 d, e (medial). Some pass forwards to dorsal 6. Posterior 5 richer to postcentral gyrus, poorer to precentral gyrus; anterior 5 the reverse.

Sulcal 5 (intaparietal) has much reduced stalk and connections of all sorts, corticofugal and corticopetal; likewise paracentral dimple; supporting earlier discovery that sulcal cortex has sparser connections than corresponding gyral (except thalamocortical). Depth of intraparietal sulcus reflects functional separateness of areas 5 and 7.

Midget or shallow lesions show as widespread connections as inclusive lesions.

181. *Observations on neuronal transport of vesicular stomatitis virus through the rhinencephalon of the mouse.* Hartwig KUHLENBECK and Maria WIENER KIRBER*, Department of Anatomy and Bacteriology, Woman's Medical College of Pennsylvania.

In 1937 and 1938 Sabin and Olitsky studied neuroinvasiveness of vesicular stomatitis virus in the mouse and demonstrated axonal as well as transsynaptic spread. With strains of VS Indiana and VS New Jersey virus supplied by Dr. Peter Olitsky we have repeated a series of intranasal instillations in order to obtain additional data concerning histopathology and distribution of cerebral lesions. We furthermore tried to ascertain whether these virus strains could be utilized as implements for a convenient and reliable method of neuronography. Young albino mice of 4–5 weeks were used after determining a suitable degree of infectivity by repeated adult mouse brain passages.

The lesions are characterized by pyknosis, a peculiar type of karyorrhexis, cytoplasmolysis, ghost cells and complete disappearance, often resulting in areas of diffuse gelatinoid degeneration. Localized meningoencephalitis with perivascular cuffing was a variable additional feature. The presence of inclusion bodies is dubious.

In prosencephalon olfactory bulb, olfactory tubercle, anterior and posterior piriform lobe, amygdaloid nucleus, fundus striati, septum, cornu ammonis, hypothalamus and epithalamus displayed lesions, occasionally spreading to thalamus. Spread into reticular formation of brain stem, also affecting additional structures, was the probable cause of death.

Although these virus lesions provide data for evaluation of neural pathways and functional systems, the numerous variables involved, causing an uneven and capricious distribution, appear to limit the usefulness of this method.

150. *Autonomic neuroeffector formations.* Albert KUNTZ, Department of Anatomy, St. Louis University School of Medicine.

In successfully impregnated silver preparations, arteries appear to be invested with a neural plexus in the adventitia and a deeper one that is located in relation to the media. The deeper plexus consists predominantly of sympathetic fibers, since it undergoes almost complete degeneration following postganglionic sympathetic denervation of the artery. Most of its fibers lie in a narrow zone at the surface of the media. They are arranged in a meshwork of slender bundles from which some fibers extend parallel to more superficial circular muscle fibers. Discrete nerve endings in the media could not be demonstrated with certainty. In preparations of the gastro-intestinal tract, the plexiform neural structures associated with the smooth muscle in general lie parallel to the muscle fibers,

but frequently give off lateral branches that cross muscle fibers obliquely or at right angles. The plexiform neural structures associated with gland tissue are intimately related to the gland cells, but discrete nerve endings are not apparent. The hypothesis that autonomic neuroeffector mechanisms require no discrete nerve endings is supported. Inclusion of cellular elements, such as the interstitial cells of Cajal, in the neuroeffector formation is not indicated.

140. *A quantitative study of the brain, of its parts and of the spinal cord in an inbred race of rabbits (race X).*[1] Homer B. LATIMER, Department of Anatomy, University of Kansas, and Paul B. SAWIN, Roscoe B. Jackson Memorial Laboratory.

The weights of the brain, its 4 divisions and of the spinal cord were studied in 100 rabbits. Fifteen of the males and 20 of the females were heterozygous for the dwarf gene.

All measurements are greater and more variable in the females but are larger as percentages of the body weight in the males. Of the total brain weight, the prosencephalon comprises 67%; mesencephalon, 8%; cerebellum, 15% and medulla, 10%. The cord weight is 43% of the brain weight in the males and 46% in the females, and its length is 61% of the body length.

Although the parts of the female nervous system are more variable, they are better correlated. The correlations vary from + 0.979 to + 0.286, the lowest being with the midbrain.

The dwarf gene reduces body size more than the size of the nervous system, and all are reduced more in the females than in the males. The weights of the mesencephalon and medulla are reduced more than the prosencephalon and cerebellum, and the proportions of the heterozygous brains are more constant as evidenced by their percentages of brain weight.

[1] This investigation was supported (in part) by research grant PHS C-2810 from the National Cancer Institute, National Institutes of Health, Public Health Service.

77. *Hormonal and nutritional influences on the male reproductive system.* James H. LEATHEM, Bureau of Biological Research and Department of Zoology, Rutgers University.

Adult male rats exhibited no alteration in testis size or in spermatogenesis when fed a protein free diet for 30 days. Furthermore, 0.25 mg of testosterone propionate daily maintained spermatogenesis in hypophysectomized rats fed a diet lacking protein. Protein free feeding for 90 days induced a varied degree of testis atrophy in normal rats but seldom abolished spermatogenesis despite a marked reduction in pituitary gonadotrophin content. Seminal vesicle weight, however, reached castrate levels. To further evaluate the need for dietary protein in testis function, adult male rats were protein depleted for 30 days, hypophysectomized and then fed either a protein free diet or a diet containing 18% casein for 20 days. Hypophysectomy decreased testis water, total protein and percentage protein, the protein changes being accentuated in rats fed the protein free diet. Histologically, sudanophilic lipid accumulated in the spermatogonia but only slight changes were noted in alkaline phosphatase and PAS preparations. Similar

groups of hypophysectomized rats were given 0.25 mg of testosterone propionate daily. Testis weight and seminal vesicle responses were improved by feeding protein but testis water and protein concentrations were comparable regardless of diet. The androgen maintained spermatogenesis and alkaline phosphatase. Lipid accumulation was prevented in rats fed protein but some spermatogonial lipid appeared when 0% casein was fed.

[1] Supported by USPH Grant A-462.

99. *Quantitative relationships between dietary iodine and sodium chloride in the induction of goiter in the C3H mouse.* C. P. LEBLOND, H. ISLER* and A. AXELRAD*, Department of Anatomy, McGill University.

To determine the effect of sodium chloride on the iodine requirement of the mouse, 174 C3H (Heston) male mice were divided into 4 groups fed diets supplying 0.13, 0.4, 1 and 3 μg of iodine per animal per day respectively. Each group was further divided into 4 subgroups receiving 0, 0.1, 1.0 and 3.0% sodium chloride mixed with the respective diets.

At autopsy 67 days later, thyroids were found to be enlarged 8 to 12 times in the animals ingesting 0.13 μg of iodine per day, the size increasing with the amount of sodium chloride in the diet. Thyroid enlargement was completely inhibited at levels of 0.4 μg or more of iodine per day whether or not sodium chloride was added to the diet.

Histologically, the thyroids were strongly activated in all animals ingesting 0.13 μg of iodine daily. At levels of 0.4 and 1.0 but not 3.0 μg of iodine, the degree of thyroid activation was increased by sodium chloride.

In general, the accumulation of radio-iodine by these thyroids was inversely related to the iodine intake of the animals. At each level of iodine intake, the radio-iodine accumulation was increased by ingestion of sodium chloride.

Thus, the iodine requirement of the mouse — as judged by the maintenance of normal structure and function of the thyroid gland — varies over a wide range depending on the amount of sodium chloride in the diet.

190. *The effect of anoxia upon the lipemia-clearing property of heparin.*[1] Virgil S. LeQUIRE, Lee M. WORLEY* and Mary E. GRAY, Department of Anatomy, Vanderbilt University.

In a study of the enzymatic nature of the lipemia-clearing effect of heparin in dogs, it was observed that anoxia alters this effect *in vivo*. Lipemias were produced by feeding olive oil 3 to 4 hours before the time of the experiments. Optical density was measured by means of a Beckman Spectrophotometer, Model B.

It has been reported that in the normal lipemic dog, a standard dose of heparin (30 mg.) will cause a reduction in the optical density of the plasma of 77 per cent of the total amount possible within 5 minutes (LeQuire, Gray and Cobb, 1953). Anoxia produced by rebreathing previous to heparin injection, inhibits this clearing response to a degree apparently relative to the degree of anoxia. Intravenous sodium cyanide which is presumed to produce a cytotoxic anoxia, also inhibited the lipemia-clearing response to heparin. The quantitative relation-

ship between the cyanide dosage and the degree of inhibition was more apparent in these experiments. Intravenous 2,4-dinitrophenol did not inhibit the lipemia-clearing response to heparin. No effects similar to these were observed in *in vitro* experiments.

It is suggested that the production of lipemia-clearing factor in response to heparin is dependent on oxidative enzymes at a cellular level.

[1] Supported by a grant from Eli Lilly and Co.

50. *The reestablishment of the neurosecretory pathway following total hypophysectomy in the rat.*[1] Theodore F. LEVEQUE*, Department of Anatomy, University of Colorado School of Medicine. (Introduced by Ernst Scharrer)

A generally applicable explanation has never been advanced for the fact that experimental removal of the posterior pituitary does not necessarily result in permanent polyuria. Some investigators have attempted to implicate the "pituicytes" in the pituitary stalk. It has been shown, however, that these cells do not elaborate the posterior lobe hormones.

An explanation for the lack of development of permanent diabetes insipidus could possibly be found in the regeneration of nerve fibers with a concomitant reestablishment of a storage and liberation center for the hormones elaborated by the cells of the supraoptic and paraventricular nuclei. Such a regeneration appears to take place (Stutinsky, '53). In an effort to study this phenomenon in more detail, young adult white rats were kept from 5 to 8 weeks following total hypophysectomy. Subsequent to this period, the proximal end of the cut stalk has regenerated into a posterior pituitary-like body within which an accumulation of neurosecretory material can be demonstrated. It is known that the neurosecretory material and the hormones cannot coexist independently. If, therefore, these animals are dehydrated and the accumulated neurosecretory material disappears, it could be concluded that the regenerated stump is again physiologically functional. Experiments completed thus far indicate that the process of dehydration does indeed result in the depletion of some animals, but not of others.

[1] ONR Contract Onr-231-VIII.

51. *Studies on the mechanism of disc herniation.*[1] H. R. LISSNER* and F. Gaynor EVANS, Department of Anatomy and Department of Engineering Mechanics, Wayne University.

The influence of static vertical and transverse (bending) loading on lumbar disc herniation has been studied in intact lumbar spines from fresh and embalmed bodies of adult male individuals. The specimens were statically loaded to failure at speeds of 0.050 and 0.071 inches/minute in a 5000 pound capacity Riehle testing machine calibrated to an accuracy of $\pm 1\%$. The bending studies were made with a specially designed apparatus by means of which the influence of bending of the spine in any plane could be tested. The effect of the applied load upon the lumbar discs was determined by nucleograms taken before and after testing as well as by macroscopic examination of the sagittally sectioned spine. The deflection occurring in the specimen during a test was measured and a load-deflection

curve plotted by an automatic stress-strain recorder attached to the testing machine. The ultimate compressive strength of individual lumbar intervertebral discs, both fresh and embalmed, was tested to determine the influence of embalming upon the strength of the discs. All specimens were moist when tested. The results obtained by the various tests will be illustrated and discussed.

[1] Supported by a research contract with the Aero Medical Laboratory, USAF, and (in part) by Research Grant A-377(C4) from the National Institutes of Health, United States Public Health Service.

178. *Study of the innervation of Clarke's nucleus in the cat.*[1] Chan-nao LIU, Department of Anatomy, University of Pennsylvania.

It has been stated that Clarke's nucleus receives its afferent fibers only from the dorsal roots. If so, this nucleus should be suitable for testing the effects of dorsal root section upon the intact neurons of Clarke's nucleus and supply data as to the mechanisms of transneuronal degeneration. Experiments to establish distribution of spinal afferents in this nucleus have been made. Each dorsal root was severed near the ganglion and after 4 to 7 days degeneration was determined by Nauta's technique which selectively stains degenerating axoplasm. Clarke's nucleus receives extensive ipsilateral innervation from a single dorsal root. Innervation is maximal at level of entrance of spinal root and gradually diminishes over as many as 11 segments of the nucleus. For example, dorsal root T10 supplies all of the nucleus except the two or three most rostral and caudal segments, L7 supplies the lower 8 segments of the nucleus. The extensive overlap of afferent supply indicates the need for extensive dorsal rhizotomy in the study of effects of denervation on the cells of this nucleus.

Preliminary experiments indicate that complete unilateral section of dorsal roots caudal to T10 results in chromatolytic changes in some cells of the ipsilateral Clarke's nucleus.

[1] Supported by a grant from U. S. Public Health Service.

21. *The effect of prolonged fixation with osmic acid on the electron microscopy of lung tissue.* Frank N. LOW, Department of Anatomy, Louisiana State University School of Medicine.

Small pieces of lung tissue were fixed in buffered osmic acid (Palade) for periods varying from a few minutes to more than 50 hours. Most structural features remain unchanged after fixation times up to 4 hours. The basement membranes of endothelium and epithelium may or may not be osmicated. Some fragmentation may be present in endothelium and epithelium, marking the beginning of cytoplasmic dissolution. After 17–20 hours fixation endothelial and epithelial cytoplasm have dissolved in most areas, but the basement membranes of both are intact and heavily osmicated. At this stage blood leucocytes and alveolar epithelial cells are represented by naked nuclei. Alveolar macrophages are more resistant but show cytoplasmic thinning and fragmentation. After 40–50 hours

most structural identities and relationships are obscure because of extensive fragmentation. The dissolution observed after prolonged fixation with osmic acid is most evident in thinly attenuated cytoplasmic sheets exposed to free surfaces. It is less complete in rounded up free cells and least so in cells closely packed together.

19. Dust concentrations in mammalian lungs as clews to non-hemic fluid flow from alveoli into lymph vessels.[1] Charles C. MACKLIN, Department of Histological Research, University of Western Ontario.

Although most of the dust that reaches the mammalian alveoli is eliminated via the glottis, a small fraction aberrantly enters the lung tissue and some of this reaches the hilar nodes and beyond. The portal of entry is spongy tissue particularly in the walls of transitional bronchioles and stems of respiratory bronchioles but also upon pulmonary venules and arterioles and even in subpleural tissue. These inlets of lymph vessels (outlets of alveoli) apparently receive the overflow of alveolar surface fluid. Much of this fluid is normally vaporized and exhaled but the remainder is presumably eliminated as lymph. Thus is provided a means of escape for this redundant fluid from the lungs whereby the alveoli are guarded from edema and the bronchial tree from flooding. The lymph drainage of the lungs, sometimes copious, is thus explained. In percolating through the epithelium of the airway-outlets and around the reticuloendothelial receptors of these initial lymph canals the fluid relinquishes much of its dust content as fine phagocytized particles. It is suggested that these accumulations of dust reveal the secret of the origin of probably most of the lymph of the lung.

[1] For this and other research herein recorded by me, assistance was furnished by the National Cancer Institute of Canada and the National Research Council of Canada.

115. Degeneration and regeneration in skeletal muscles of vitamin E deficient hamsters as a basis for bioassay of alpha-tocopherol and other anti-dystrophic substances.[1] Karl E. MASON and William T. WEST*, Department of Anatomy, The University of Rochester School of Medicine and Dentistry.

Syrian hamsters reared from weaning on vitamin E deficient diets manifest, after about 60 days, rather widespread microscopic lesions of muscular dystrophy. Interspersed among normal fibers are numerous fibers showing progressive segmental fragmentation and necrosis and also many others showing successive phases of regeneration from nucleated fragments of degenerated segments. Effective vitamin E therapy arrests the dystrophic process and, after a period of 5 to 10 days, results in disappearance of degenerating fibers such that only regenerating and normal fibers are demonstrable microscopically.

In more than 150 hamsters serving as control and test animals in evaluating the anti-dystrophic activity of alpha-tocopherol and related compounds, comparisons have been made between samples of sacrospinalis muscle obtained by biopsy before therapy, and at sacrifice after therapy; 12 other individual muscles have been routinely studied and evaluated with respect to the relative extent of de-

generation and repair. These results form the basis for a proposed bioassay procedure for anti-dystrophic compounds. The results obtained to date, indicating that when administered orally beta-tocopherol, alpha-tocopherylhydroquinone and alpha-tocopherylquinone possess approximately one-half the anti-dystrophic activity of alpha-tocopherol, and that certain esters of the hydroquinone and other oxidation products of tocopherol are inactive, will be discussed.

[1] Aided by Grant-in-aid from the Muscular Dystrophy Associations of America, Inc.

194. Comparison of skin and colonic temperature reactions in rats subjected to total body stress with those due to localized thermal stimuli.[1] L. C. MASSOPUST, Jr.* and W. R. MC CRUM, Department of Anatomy, University of Colorado.

When rats are placed in a cold environment they respond by peripheral vasoconstriction, as indicated by decrease in skin temperature, without appreciable change in colonic temperature. If these animals are given a vasodilator drug, peripheral vasodilation occurs, accompanied by a fall in colonic temperature. When anesthetized rats were subjected to a localized cold stimulus while in environments maintained at 24 to 26°C., a parallel decrease in skin temperature and colonic temperature was regularly observed. When these animals were subjected to a localized heat stimulus at normal environmental temperatures, there were parallel and simultaneous increases in skin and colonic temperatures.

On the basis of these experiments it appears that, while peripheral vasoconstriction plays an important role in the reaction to cold stress, under normal environmental conditions and while generalized peripheral vascular reactions to localized thermal stimuli are as expected, body temperature responds paradoxically in the latter situation. The change in body temperature may reflect an increase in metabolic activity which is due to nervous stimulation of, or simple thermal changes in, the hypothalamus.

[1] Supported by grants from Armour and Company and the United States Public Health Service.

184. Gross observations of peripheral nerve regeneration in the white rat with small doses of the drug piromen.[1] A. W. MC CULLOUGH, Department of Anatomy, University of Arkansas School of Medicine.

Litter-mate pairs of weanling Sprague-Dawley rats were operated upon, under nembutal anaesthesia, the right sciatic nerve being cut through half to two thirds of its cross dimension with fine scissors. The experimental member of each pair was given piromen in a sodium lactate R soluton while the control member received the lactate solution in the same dilution without the piromen, as a placebo. Dosage varied from 0.25 to 0.75 μg/kg body weight. Injections were made subcutaneously adjacent to the operative site on the day of operation and on every fourth post-operative day for periods of from two to five weeks. Both members of a pair were sacrificed at post-operative periods ranging from two weeks to six months.

Gross findings include (1) the frequent presence of neuromata at the site of nerve section in the control animals and a complete absence of such structures in the experimental animals; (2) markedly less subcutaneous and intermuscular fibrosis in the experimental animals as compared to the controls for periods up to three months after operation; (3) better return of function in the operated leg of the experimental animals, a difference noticeable three weeks after operation.

Microscopic findings will be reported later in relation to these gross observations.

[1] Piromen and placebo were provided by the Baxter Laboratories, Morton Grove, Illinois, Dr. William F. Windle, Scientific Director.

139. Blood supply of the caudate and quadrate lobe of the human liver.[1] Nicholas A. MICHELS, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

A statistical estimate based on extrahepatic dissection of 50 specimens. Arteries entering caudate lobe were derived from (1) right hepatic or aberrant right hepatic (60%) via branch that descended behind portal vein giving off bifurcating twigs to caudate process before ending in papillary process with grapevine-like distribution of twigs; (2) right and left hepatic (14%), former supplying branches to caudate process, latter to papillary process; (3) middle hepatic derived from right hepatic (6%); (4) right, left and middle hepatic (4%); (5) right and middle hepatic (8%); (6) solely from left hepatic or hepatic (8%). Caudate branches varied from 3–10. Prevailingly, they were short, tiny, communicating, subcapsular branches that bifurcated before entry.

Quadrate lobe (part of medial segment of left lobe as demonstrable in 150 vinyl acetate plastic casts of human livers made at this Institute by Healey and Schroy) was supplied mainly by the middle hepatic derived in about equal proportion from right hepatic (50%) or left hepatic (44%) and in 6% from other sources (celiac, hepatic, gastroduodenal, right gastric). In umbilical fossa middle hepatic intermittently and at different levels gave off small branches (3–7) to left side of quadrate lobe and to floor of fossa. Distally it supplied branches to falciform ligament which communicated with branches from internal mammary. In instances, it gave off branches to left lobe, as comparably, the left hepatic supplied branches to quadrate lobe. Subcapsular terminals of middle hepatic frequently anastomosed with one another and with branches from the right or left hepatic.

[1] Aided by a grant from the American Philosophical Society.

140. The lumbar vertebral column at rest and in abduction. Meryl MILES and Walter SULLIVAN, Department of Anatomy, University of Wisconsin.

The lumbosacral and lumbar intervertebral joints were studied in 20 young adults. Abduction, as used here, refers to both the lateral flexion and the torsion. Photographs and x-rays were made of each subject standing at rest; abducted to the right and to the left. The relation of the sacrum and each lumbar vertebra to the horizontal was observed. The comments that follow are based on this relationship.

At rest displacements from the vertical and/or torsion could be seen in 13 columns. The maximum deviation of the cephalic border of the sacrum from the horizontal was 5°; that of the first lumbar vertebra was 9°; that of any single vertebra was 6°.

In abduction, displacement in the lower segments is usually contralateral. Only 5 individuals showed homolateral movement at all 5 joints and in only 2 of these was it bilateral. Abduction was minimal at the lumbosacral joint. It was usually maximal at 1 or 2 of the 3 upper joints but occasionally it was maximal between L4 and L5. For any single segment the movements to the right and left were equal or unequal in about the same frequency. The total maximal displacement in any one individual was 36°; the minimal was 6°.

5. *Pattern perception after implantation of dielectric plates in the visual cortex.*[1] Nancy MINER* and R. W. SPERRY, Division of Biology, California Institute of Technology.

It has been found that dense implantation of tantalum wires throughout the visual cortex or extensive subpial slicing of the visual cortex in criss-cross patterns fails to produce any marked disturbance of detailed pattern perception in the cat. On the possibility that implanted wires and knife scars may not distort significantly the configuration of DC flow in the cortex, another test was conducted in which thin plates of mica were inserted vertically into the visual area. Most of the mica plates invaded the white matter for variable distances. They were placed in patterns designed to spare the optic radiation fibers but to effectively distort tangential direct current flow across the cortex. In 3 cats so treated, visual pattern discrimination was only slightly impaired and rapidly returned to approximately the preoperative level. The impairment was greater in two other cases, but in these latter two visual discrimination returned gradually in the course of 3 months to near the preoperative level. The degree of functional impairment seemed to be correlated primarily with the degree of invasion of the white matter and secondly with cortical damage. Taken together the findings fail to indicate a dependence of visual pattern perception upon the massive DC currents in the cortex as conceived in electrical field theory.

[1] Supported in part by funds from the National Institute of Neurological Diseases and Blindness.

6. *A reappraisal of the formation of the hair germ in hair follicles.* William MONTAGNA and Herman B. CHASE*, Department of Biology, Brown University.

The belief that hair follicles during the transition from the active to the quiescent state set aside a "hair germ" is not tenable. Chase (1954, Physiol. Rev.) has recently compiled abundant evidence against this view, and our observations on the effect of X-rays on human hair follicles are of particular interest. After the scalp is X-irradiated with 750 r, the entire matrix of the bulb degenerates. The upper bulb degenerates more gradually and becomes reduced to an attenuated cord of cells. Since a club fails to form, the hair is shed and the whole follicle becomes a solid cord of cells composed of cells from the ex-

ternal sheath. As the lower part gradually degenerates, the cord is shortened and its base retreats within the dermis. The dermal papilla, always in contact with the base of the cord, retreats upward with it. After 5 to 7 weeks the base of the follicle and its associated papilla, rest halfway up in the dermis. At this time the cells at the base of the cord in contact with the dermal papilla begin to proliferate and they grow around the dermal papilla. This is the onset of recovery and growth of a new hair bulb. These observations focus attention on the external sheath in the upper one half of the follicle as the source of the "hair germ." The lower half of the hair follicle, including the bulb, is a transient structure which must be formed anew from the cells of the external sheath at the onset of each hair generation. During the periods of growth and rest, the upper one half of the external sheath is the only permanent portion of a hair follicle. We believe that the events in normal catagen are essentially similar to those outlined above.

172. *Effects of uterine distention during the estrous cycle on ovine reproduction.*[1] W. W. MOORE* and A. V. NALBANDOV, Department of Animal Science, University of Illinois.

Uterine distention alters the estrous cycle through a neurogenic mechanism. Experiments were designed to determine the degree of uterine distention necessary to cause aberrant cycles and the effects of distention initiated at various stages of the estrous cycle.

Distention was produced by suturing a plastic bead (2, 4 or 8 mm in diam.) into the uterine lumen during metestrus, diestrus, or proestrus of normal ewes.

Two-mm beads had no apparent effect on the length of the estrous cycle. Also 8-mm beads introduced during proestrus had no significant effect on cycle length (17.4 ± 0.80 days), but increased the variability. However, 4- and 8-mm beads introduced during metestrus caused a significant decrease in cycle length (12.8 ± 1.77 and 13.0 ± 1.33 days respectively) compared to the normal of 16.3 ± 0.10 days. Eight-mm beads introduced during diestrus caused a significant increase in estrous cycle length (22.8 ± 1.21 days), but denervation of the distended portion of the uterus resulted in cycles of normal length (17.0 ± 0.55 days).

The effects on cycle length which follow uterine distention vary with the time of the cycle at which distention is started, and support the hypothesis that changes in the cycle result from alteration of the endocrine activity of the anterior pituitary gland through a neural or neurohumoral mechanism.

[1] Supported by a grant from the U. S. Public Health Service.

173. *The distribution and relative number of osteoclasts in the mandibular condyle in guinea pigs of different ages.*[1] Hugh I. MYERS* and Wayne L. BRUNE, technical assistance by Viola FLANAGAN*, Department of Histology and Pathology, University of Kansas City, School of Dentistry. (Introduced by Strand W. Chase)

The mandibular rami from normal male guinea pigs ranging in age from two to fifty-eight weeks were sectioned and studied histologically. Sagittal, frontal

or cross sections were made. The sections showed the tissue layers characteristic of mammalian mandibular condyles. These consisted of an outer dense fibrous layer, a cellular fibrous layer, a hyaline cartilage layer, and then the bone which continues into the neck and ramus. As visualized by most investigators, ramal height growth is accomplished by proliferation of the cellular fibrous layer, this is converted to hyaline cartilage which in turn becomes calcified, destroyed and replaced by bone. Since the condylar head is larger than the ramus, investigators have assumed bone resorption occurred in the neck region. Growth in mandibular length is accomplished by bone deposition on the ramal posterior border with simultaneous bone resorption on its anterior border.

Osteoclasts were found internally adjacent to the calcified cartilage spicules, under the periosteum on the neck medial and lateral surfaces, and the anterior ramal border below the condyle.

The number of osteoclasts seen on every tenth section in these regions was tabulated and averaged. Sagittal, frontal and cross sections all showed a constantly decreasing number of osteoclasts per slide with increasing age from six through fifty-eight weeks. The osteoclast count taken from animals two to six weeks of age show more variability.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Kansas City, School of Dentistry (NR 180 031).

151. Innervation of the ocular blood vessels in the cat. Leonard M. NAPOLITANO*, Department of Anatomy, St. Louis University School of Medicine. (Introduced by Albert Kuntz)

Cats were subjected to surgical removal of either the ciliary or the superior cervical sympathetic ganglia. Twenty to 28 days were allowed for degeneration after which the entire orbital contents of 9 cats were serially sectioned at 20 microns and stained with a modified Gros-Bielschowsky, or Holmes' silver technique, or with hematoxylin and eosin.

Removal of the ciliary ganglion did not result in complete parasympathetic postganglionic degeneration within the eye due to accessory ganglia in the short ciliary nerves both without and within the sclera. This operation resulted in no observable changes in the innervation of the ocular vessels.

Superior cervical ganglionectomy resulted in degeneration of most of the nerve components to the blood vessels, but a small number of nerve fibers remained intact. Some of these undoubtedly were afferent. Some may have arisen from accessory sympathetic ganglia. Removal of the superior cervical ganglion probably results in degeneration of few if any sensory fibers of the ocular blood vessels.

No discrete nerve endings were observed in the blood vessels. Most of the nerve fibers are involved in a plexus located at the surface of the media.

63. Observations on the efferent connections of the thalamus. Blaine S. NASHOLD*, John HANBERY*, Jerzy OLSZEWSKI, Department of Neurology and Neurosurgery, McGill University and the Montreal Neurological Institute.

Extensive cortical ablations and focal lesions of the striatum were made in cats and monkeys, and the degeneration of the thalamic nuclei was studied in Nissl

stain after survival periods of two months or longer. Degeneration in the thalamus does not follow the "all or nothing" principle. Between the extremes of entirely normal appearance and complete degeneration with loss of ganglion cells and heavy gliosis there exists an array of intermediate changes. Their significance in the organization of thalamic efferents will be discussed.

Lesions restricted to the caudate or putamen did not result in degeneration in the thalamic nuclei. Lesions of the caudate which involved the adjacent portion of the internal capsule were followed by extensive cell loss and gliosis which involved the oral portion of the reticular nucleus, the nucleus ventralis anterior and the oral portions of the intralaminar nuclei.

Cortical removals consisted of hemidecortication, either complete or limited to the isocortex. In addition to degeneration of the specific projection nuclei, marked changes were present in the intralaminar nuclei. The changes were observed after removal of the isocortex alone with varying amounts of damage to the underlying white matter. In complete hemidecortication the extent of degeneration of the intralaminar nuclei was only slightly modified. The details of these findings will be presented and their significance discussed.

60. *Terminal distribution of some afferent fiber systems in the cerebral cortex.* Walle J. H. NAUTA, Department of Neurophysiology, Army Medical Service Graduate School. (Assisted by David G. Whitlock)

The intracortical distribution of degenerating axons was studied following (a) lesions of various specific thalamic nuclei in the cat; (b) small ipsilateral cortical lesions, mostly in the striate and auditory I areas of rat and cat; (c) smaller or larger contralateral cortical lesions in rat, cat, and monkey. All cases of thalamic lesion showed profuse terminal degeneration, mainly of the pericellular type, in layers IV and III of the corresponding cortical area; and fewer degenerating fibers (probably corresponding to Lorente de No's non-specific cortical afferents) were observed to continue and arborize into layers II and I. Ipsilateral intracortical and corticocortical fibers show an extremely complex arrangement; such fibers apparently terminate in all cell layers, but most profusely in layers VI to III. Degenerating callosal afferents likewise could be followed to all cell layers; the largest proportion of such fibers appears to distribute to layers VI to III, while only few could be traced into II and I. Both ipsilateral and callosal association fibers exhibit relatively few pericellular terminations.

55. *Recovery of spermatogenesis in rats treated with nitrofurans.*[1] W. O. NELSON, E. STEINBERGER* and A. BOCCABELLA*, Department of Anatomy, State University of Iowa.

Earlier reports have described the spermatogenic arrest that occurs in rats treated with nitrofuran compounds (Nelson and Steinberger, Anat. Rec., *112*: 367, 1952). The effect is apparent within a few days and has been maintained for more than fourteen months without interfering with other physiological activities or with the capacity for spermatogenic recovery. In otherwise untreated animals recovery is manifest twenty days after cessation of treatment, is well

under way at thirty-five days and is complete at sixty days. If animals are injected with estradiol or testosterone during the period of nitrofuran treatment testicular size is greatly diminished, but recovery is markedly accelerated. Thus recovery begins about the sixth day after withdrawal of both nitrofuran and hormone, and is complete by the thirty-fifth day. When animals received nitrofuran, estrogen and gonadotrophin (usually pregnant mare serum) marked depression of testicular size did not occur and recovery took place as in animals receiving nitrofuran only. Studies on glucose utilization by testes from nitrofuran treated rats (Featherstone, Weldon and Nelson, Federation Proc., 1954) show an interference with the terminal oxidative stages, notably pyruvate utilization. When treatment was withdrawn recovery of this function occurred at a rate parallel to the recovery of spermatogenesis. In animals treated with estrogen and nitrofuran recovery of the capacity for pyruvate utilization was accelerated.

[1] Aided by a grant from United States Public Health Service, R.G. 1778.

90. *Mitotic abnormalities in hematopoietic cells following splenectomy.* Susumu OHNO*, Jeremy P. WARD* and Riojun KINOSITA*, Departments of Infectious Diseases and Pathology, School of Medicine, University of California at Los Angeles and Medical Research Institute, City of Hope Medical Center. (Introduced by Daniel Pease)

Howell-Jolly bodies were observed in erythrocytes of splenectomized rats and appeared to be composed of two even-sized bodies coupled together. Structures with the same characteristics were also observed in the leukocytes of peripheral blood and in the normoblasts, myelocytes, megakaryocytes and monocytic cells of the bone marrow of these splenectomized animals. Some cells, belonging to these four kinds of bone marrow cells, showed mitotic abnormalities. A pair of chromosomes was dislocated from the metaphase plate and remained in the equatorial region as an unseparated pair, while the main chromosome group separated and moved to the poles as mitosis progressed to ana- and telophase. The pair of deserted chromosomes was included as a coupled body in the cytoplasm of one of the separating daughter cells. The coupled bodies were persistent in the erythroblastic cells after the resolution of nuclei. Absence of the spleen appeared to cause a disturbance in the karyokinetic control which affected many types of hematopoietic cells. As a result of these observations it is believed that the Howell-Jolly type of cytoplasmic inclusion occurs in other hematopoietic cells besides cells of the erythroblastic series and that such structures are a result of mitotic abnormalities.

33. *The prenatal development of the human centrale.* Ronan O'RAHILLY, Department of Anatomy, Wayne University College of Medicine.

Fifty human embryos and fetuses ranging from 16 to 195 mm. C.R. length were examined in the region of the centrale carpi by serial sectioning. The centrale was found as a free element in specimens between 16 and 19 mm. inclusive. A variable degree of fusion with the scaphoid had occurred in specimens from 22 to 49 mm.; at later stages, although the centrale could be identified in many instances, fusion was complete. Fusion commenced palmarly, occurred

before cavitation, and involved obliteration of a homogeneous interzone. No evidence of disintegration of the centrale was found in this investigation. Particular attention was given to the relations of the centrale, on which its homologization must depend. Serial projections at early stages revealed the relationship to the scaphoid, capitate, and trapezoid; proximity to the lunate, extent of contact with the trapezium, and the degree of exteriorization were variable. The findings were compared with those in adult cases of free centrale and bipartite scaphoid, and also with comparative primate data.

129. *The ventricular system in hydrocephalic rat brains produced by a deficiency of vitamin B_{12} or of folic acid in the maternal diet.*[1] M. D. OVERHOLSER, J. R. WHITLEY*, B. L. O'DELL* and A. G. HOGAN*, Departments of Anatomy and Agricultural Chemistry, University of Missouri.

Hydrocephalic rats were obtained from dams fed a diet deficient in B_{12} or folic acid.

In 25 hydrocephalic brains from day-old rats the cerebral aqueduct was closed in 2 and abnormal in shape and size in 23. The fourth ventricle was normal. Controls showed a group of ependymal, secreting cells in roof of third ventricle and aqueduct which were partially or completely missing in hydrocephalics.

Fifty brains from litter mates of hydrocephalics appeared grossly normal; sections showed 15 normal; 12 had partially closed aqueducts with the special ependymal cells partially missing and 4 of these had moderate hydrocephalus; 23 showed slight aqueduct constriction, normal ependymal cells and no hydrocephalus.

Twenty-one embryos from B_{12} depleted dams which had produced 3 consecutive hydrocephalic litters were sectioned; 12 at 16 or 17 days of gestation had normal brains; 9 at 18 days were hydrocephalic. Six of the latter had hydrocephalus with aqueducts completely closed. The special ependymal cells were missing while prominent in controls. Three had slight hydrocephalus with special cells partially missing.

Occlusion of aqueduct occurs between gestation days 16 and 18, due to absence of special group of cells in roof of aqueduct and third ventricle. Secretion from these cells prevents collapse of aqueduct before cerebrospinal fluid is formed. Usually before birth cerebrospinal fluid pressure from choroid plexus distends ventricles and partially reopens the aqueduct.

[1] Aided by a grant from the U. S. Public Health Service.

130. *Electron microscope observations of interneuronal and neuromuscular synapses.* George E. PALADE, The Rockefeller Institute for Medical Research. (Assisted by Sanford L. Palay[1])

Central and peripheral synaptic regions were examined with the electron microscope in the rat. In the central nervous system (cerebellar cortex and the neuropyl of the medulla oblongata) typical end-foot or "bouton terminal" appearances were occasionally encountered in contact with dendrites. In the axon ending an accumulation of mitochondria and small vesicles (300–500 Å) was found, whereas the dendrite showed fewer mitochondria and vesicles in a rather fibrillar cytoplasm.

The axon and dendrite appeared to be separated by their respective plasma membranes, which at the level of closer contact were denser and thicker. The space between the synaptolemmae was ~ 200 Å. Similar cytoplasmic and membrane appearances were encountered in the glomeruli cerebellosi and the molecular layer. As for epidermal cells, the thickening of the apposing membranes at the level of the synapse may represent a zone of intimate adherence.

The neuromuscular synapses were studied in rat skeletal muscles. The axon endings had the same cytoplasmic appearance as in central synapses. The sarcoplasmic sole showed large accumulations of mitochondria and small granules. The axon and the muscle fiber were separate. The membrane of the latter showed a number of deep, narrow infoldings ~ 700 Å thick and 0.2–0.4 μ apart, within which thin, dense lamellae were sometimes encountered (subneural apparatus of Couteau). No teloglial component was apparent between the nerve and the muscle fiber.

[1] Department of Anatomy, Yale University School of Medicine.

111. *Electron microscope study of the cytoplasm of neurons.* Sanford L. PALAY,[1] The Rockefeller Institute for Medical Research. (Assisted by George E. Palade)

Representative neurons were examined in thin sections of sympathetic and dorsal root ganglia, medulla oblongata, and cerebellar cortex. Tissue obtained from living, anesthetized rats was fixed in 1–3% buffered osmium tetroxide and embedded in n-butyl methacrylate. In electron micrographs of such neurons the most conspicuous cytoplasmic features are compact masses of Nissl substance. Like basophil material elsewhere, Nissl bodies comprise two components: a thin membranous endoplasmic reticulum arranged as more or less parallel, anastomosing tubules or flattened vesicles, upon and between which lie clusters of punctate granules, 10–30 mμ in diameter. The cytoplasm between Nissl bodies contains numerous mitochondria, rounded lipid inclusions, and fine filaments 6–10 mμ in diameter and of indefinite length. In addition, there is a second system of membranes distinguished from that of Nissl bodies by narrower lumina and tighter packing of the membranes and by absence of granules. Intermediate forms between the two membranous systems exist. Nissl substance extends into dendrites for a short distance. Beyond this point dendrites and axons have similar structures. The endoplasmic reticulum loses its associated granules and becomes drawn into parallel slender strands among which are occasional mitochondria and numerous fine filaments like those in the perikaryon. A collection of mitochondria and small vesicles (20 mμ in diameter) fill the axon termination. The cell membrane at this site is thickened and denser than elsewhere.

[1] Permanent address: Department of Anatomy, Yale University School of Medicine.

18. *Some observations on certain large granules (LG) in the exocrine pancreatic cells of 14- to 17-day-old C_3H mice.* Harold F. PARKS*, Department of Anatomy, University of North Carolina. (Introduced by Charles W. Hooker)

Is the typical zymogen granule (0.5 to 1.5 μ in diameter) derived from a tiny germ that grows continually until it matures (Järvi, '40; Xeros, '51), or is it

larger at some developmental stage than at maturity (Hirsch, '32)? This conflict in the literature emphasizes the importance of the LG (1.5 to 2.5 μ diameter) here described.

There were usually only 3 or 4 LG to a cell, situated just beneath the mass of ordinary zymogen granules or in para- or subnuclear location (these were seen to change their positions freely in living cells). Sometimes a single cell, and more rarely every cell in an acinus, was filled exclusively with LG. In the latter 2 instances, LG were refractory to pilocarpine stimulation. It was therefore easy to obtain tissue in which LG were very conspicuous.

The following observations indicate that LG are not homogeneous spherules: (1) When blackened with acid haematein (Baker, '46), many were resolvable into irregular vesicles similar to certain vitally-stainable cytoplasmic structures which wax and wane in number during the process of zymogen granule restitution. (2) Osmication following Altmann's fixative darkened LG peripherally, and the internal parts of the largest ones were stainable with aniline acid fuchsin.

These morphologic and pharmacodynamic characteristics suggest (1) that LG are immature zymogen granules, and therefore (2) that *some* (not necessarily *all*) zymogen granules derive from larger precursors.

195. The effect of localized beta irradiation on the skin cycle of the rat. Jerome P. PARNELL and Joseph F. GENNARO, Jr.*, Department of Anatomy, State University of New York, College of Medicine at New York City.

Ceramic bead sources of Strontium^{90}-Yttrium^{90} and Cesium^{134} containing 2 microcuries of isotope per bead and inactive control beads were implanted subcutaneously in the ears, dorsum and venter of rats of the Long-Evans strain. The sites of irradiation were excised at 2, 4, 8, and 16 days following implantation, the beads removed, and the tissue prepared for microscopic examination.

After 2 days the tissue reaction to the initial trauma subsides in the control area which then appears normal. The area of inflammation in the irradiated site spreads concentrically to about 4 millimeters in diameter and at 8 days can be noted macroscopically by a center of cornification. Initial microscopic changes include cellular hypertrophy and increases in connective tissue and fat. Later a central zone of damage occurs within the circle of inflammation. This is characterized by destruction of the more actively growing structures in the epidermis. The damaging effects are therefore expressed differently at various stages in the cyclic growth of such elements as sebaceous glands and hair follicles. The effects produced by the different ionizing emanations are compared.

196. A statistical evaluation of cardiac measurements implemented by X-ray motion pictures. Paul R. PATEK and Samuel WEISMAN*, Departments of Anatomy and Medicine, University of Southern California School of Medicine.

Five hundred human male subjects from 18 to 70 years of age were studied. Each subject received a physical examination and a medical history was taken. Age, height, weight, blood pressure, pulse rate, thoracic measurements and vital capacity were recorded and an X-ray motion picture of the heart was made. Of

the entire group, 108 subjects met rigid qualifications which indicated no cardiac or other possible pathology. The data obtained from the selected subjects were statistically studied. A partial regression analysis was made, and equations for predicting the long, broad and transverse diameters of the heart were established.

[1] Supported by a grant from the National Heart Institute, United States Public Health Service.

67. *Replacement and differentiation of the intestinal epithelium in the Urodele.* Stanley F. PATTEN*[1] and Warren ANDREW, Departments of Anatomy, Western Reserve University and Bowman Gray School of Medicine.

A critical study of the cells composing the intestinal epithelium of the *Urodele* was made with respect to their method of replacement in the adult animal. Nine species of salamanders were utilized. A survey of the so-called intestinal glands of the *Urodele* was made with emphasis on their function and possible relationship to the crypts of Lieberkühn. Specimens of *Triturus viridescens* were given intraperitoneal injections of nitrogen mustard and colchicine and their effect on mitosis, cell migration, and differentiation was observed.

Submucosal aggregations of cells or "cell-nests" were observed in all specimens. These aggregations were found to be transient structures entirely dependent upon the functional state of the mucosa. Their function appeared to be that of epithelial replacement alone, although early differentiations of the nidal cells resulted in the formation of mucous elements. The nidal cells would seem to possess migratory properties. Normal mitotic activity within the "cell-nest" was present in 0.57% of the cells, was entirely absent after nitrogen mustard, and was not significantly altered after colchicine. Mucous cells comprised 2.8% of the "cell-nest" population and after nitrogen mustard made up 12.5%. The possible relationship of mesodermal elements to the nidal cells and the relative lack of mitotic activity is suggestive of a secondary method of epithelial replacement in the intestine.

[1] Supported in part by a U.S.P.H.S. Predoctorate Fellowship from the National Cancer Institute while at the Department of Anatomy, The George Washington University.

141. *The architecture of the normal adrenal cortex of the rat.* John E. PAULY*, Department of Anatomy, The Chicago Medical School. (Introduced by John J. Sheinin)

The adrenal cortex of the rat is composed of a continuous mass of cells tunneled by blood vessels. The three zones of the cortex can be identified by two means: (1) the arrangement of blood vessels within the continuum and (2) the relative size and appearance of the cytoplasm and nuclei of the individual cells.

The subcapsular arterial plexus gives rise to the capillaries of the zona glomerulosa. The vessels of this zone penetrate the continuous parenchyma at all angles to the capsule.

In the zona fasciculata the capillaries are parallel to each other and perpendicular to the capsule.

In the zona reticularis the capillaries dilate into irregular sinusoids which penetrate the continuum obliquely toward the medulla.

When the parallel capillaries of the zona fasciculata and the sinusoids of the zona reticularis are longitudinally sectioned, one gets the erroneous impression that these two zones are composed of cords of cells. Actually, the whole cortex is a continuous mass of cells tunneled by blood vessels.

The collagenous, fibrous capsule is continuous with reticular fibers which form the stroma. Argyrophilic fibers support the individual cells in all three layers. Occasionally collagenous fibers from the capsule project into the parenchyma along with the larger vessels. Small membranous accumulations of van Gieson positive material are often seen between the cells of the zona reticularis.

31. Observations on the gubernaculum in human embryos and fetuses.[1] Anthony A. PEARSON and Donald S. BOOTS*, Department of Anatomy, University of Oregon Medical School.

The gubernaculum was studied in a series of human embryos and fetuses stained with silver methods. The question is still raised as to whether or not the gubernaculum extends through a hiatus in the abdominal muscles. Photomicrographs will be shown demonstrating that the gubernaculum does extend through the subcutaneous inguinal ring. Superficial to this ring it fuses or is continuous with a fascia-like layer which resembles in position the deep layer of the superficial fascia. This layer extends laterally toward the thigh, inferiorly into the labioscrotal swelling where it is lost, superiorly over the abdomen where it gradually fades out, and medially over the lower end of the rectus sheath where it has attachments.

A rich network of nerve fibers supplies the lower end of the gubernaculum. These are derived from the genitofemoral and the ilio-inguinal nerves.

Strands of striated muscle fiber derived from the abdominal muscles become arranged around the lower end of the gubernaculum in anticipation of the formation of the cremaster muscle. In the female these muscle fibers extend along the gubernaculum toward the uterus. In some adults strands of striated muscle fibers can be demonstrated in the round ligament of the uterus.

[1] Aided by a grant from the U. S. Public Health Service.

32. Further studies of the kidney cortex by electron microscopy.[1] Daniel C. PEASE, Department of Anatomy, School of Medicine, University of California at Los Angeles and Sawtelle Veterans Administration Hospital.

Structures in the cortex of rat kidneys have been reinvestigated with the electron microscope, utilizing improved techniques of fixation and sectioning. Greatest attention has been paid to the glomerular capillary. So far it has not been possible to demonstrate fibrous structures in its basement membrane, even when the latter was viewed in the horizontal plane. Long, interdigitating processes of epithelial cells extended over the basement membranes, forming an external sheath. The terminal processes of these seemed definitely attached to the basement membranes and had a considerable material density suggesting a role as supporting structures. There were consistent, rather uniform spaces between processes which probably allow free percolation of capillary filtrate. The cytoplasm of endothelial cells was extensively attenuated. A regularly arranged system of pores was found

the entire group, 108 subjects met rigid qualifications which indicated no cardiac or other possible pathology. The data obtained from the selected subjects were statistically studied. A partial regression analysis was made, and equations for predicting the long, broad and transverse diameters of the heart were established.

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throughout the endothelial sheet. These pores were of nearly constant diameter (approximately 0.1 micron). At the time of this writing it seems impossible to decide whether or not these pores represent fixation artifacts, for in life a thin film of watery cytoplasm might span these gaps. Some cytological details relating to other parts of the cortex will be considered.

[1] Aided by the Los Angeles Heart Association and the U. S. Public Health Service.

187. Renal function. I. Excretion of phenolsulfonphthalein by the amphibian (Rana pipiens) kidney. Harold M. PECK, Pharmacology Section, Research Division, Sharp & Dohme Division, Merck & Co., Inc.

Richards and Barnwell ('27) described a series of experiments in which their objective was to determine whether the amphibian renal tubules actively secreted phenosulfonphthalein (PSP). Concentration of PSP in the lumen of the tubules was observed to follow perfusion of the kidney through the aorta, the portal vein, or the ureter. They concluded that the concentration of PSP observed in these tests was the result of the reabsorption of water by the nephron although tubular secretion of PSP was not necessarily disproved.

Benemid, an agent that inhibits the renal excretion of PSP (Beyer, '50), was incorporated into experiments similar to those described by Richards and Barnwell. (1) When Benemid and PSP were injected together into the lumen of the tubules by way of the ureter, concentration of the dye was not inhibited conclusively. (2) Perfusion of Benemid with PSP through the portal vein or their co-injection into the lymph sac of the frog resulted in definitely less or no apparent, concentration of PSP in the renal tubules, but (3) if PSP and Benemid were injected then into the ureters of these kidneys concentration of the dye occurred in the lumen of the nephron. (4) These results indicate that Benemid inhibited the tubular secretion of PSP but did not interfere with the reabsorption of water.

157. Functional recovery after section of the cervical sympathetic trunk in the cat.[1] Edward McC. PEEBLES*, Department of Anatomy, Tulane University. (Introduced by Adrian F. Reed)

The right cervical sympathetic trunk was sectioned in 25 cats, at levels from 0–65 mm caudal to the superior cervical ganglion. In three additional animals the sympathetic trunk was sectioned at two levels, at 10 mm and at 30 mm caudal to the ganglion. Daily observations were made to determine the earliest recovery of approximately normal function. The criteria used were right-left comparisons of the sizes of the pupils, positions of the nictitating membranes and degree of dilation of vessels in the pinnae. Indications of regeneration were checked by electrical stimulation. Observations have been completed on 15 cats with single sections; full functional recovery was found to exist after an average of one day per millimeter of regenerated nerve. This, expressed as a rate, is approximately 1.0 mm/day, the range being 0.4–1.3 mm/day; calculations were based on measurement from the section to the middle of the ganglion.

Histological preparations of this material are being studied.

Additional investigations are being made to determine the maximum gap in the trunk which can be bridged by regenerating fibers and the functional regeneration rate across such gaps.

[1] This investigation was supported by a research grant [PHS B-496 (C5)] from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service.

58. An experimental analysis of the mesencephalic nucleus.[1] J. H. PERRY* and J. C. HALEY, Department of Anatomy, Baylor University College of Medicine.

Transection of the communications between the trigeminal and facial or hypoglossal nerves results in chromatolysis of cells in the trigeminal ganglion and mesencephalic nucleus of dogs. An excess of chromatolytic cells over the number of fibers in the communications was found. This discrepancy was exaggerated further by the observation that 30–40% of the fibers in the communications were fine myelinated and unmyelinated fibers of trigeminal ganglion and sympathetic origin. In view of the difficulties using the retrograde method, lesions of the mesencephalic nucleus were placed in nine monkeys. Degenerating fibers were found in the communications between the trigeminal and facial, and also the trigeminal and hypoglossal nerves. The possibility that the tongue and mimetic musculature have proprioceptive afferents is considered.

[1] Supported by a grant from the M. D. Anderson Foundation.

47. Cytology of the adenohypophysis as revealed by pH staining. Roy R. PETERSON and Jules M. WEISS*, Department of Anatomy, Washington University.

The degree of basophilia, acidophilia, and the PAS reaction was determined for cells of the adenohypophysis of the cat, rabbit, rat and mouse. This was accomplished through the use of 2 μ serial sections in which the reactions of single cells to these staining procedures could be studied in three consecutive sections.

The PAS negative, alpha cell granules were stained with fast green up to pH 7.0, and with methylene blue down to pH 7.0. The PAS positive, beta cell granules were stained with methylene blue down to pH 4.0, and with fast green up to pH 6.0. Nongranular cytoplasmic areas in alpha cells as well as small areas of gamma cells were stained with methylene blue down to pH 4.0. In all cell types the cytoplasmic or granular basophilia was observed below pH 6.0 following ribonuclease digestion but the PAS reaction and fast green staining were unaffected.

These data are consistent with the interpretation that beta cell granules contain at least two substances: a PAS positive material which will stain with either acidic or basic dyes under the proper conditions, and ribonucleic acid. Alpha cell granules contain a more acidophilic substance which will stain with acidic or basic dyes, but contain little or no ribonucleic acid. Ribonucleic acid, not associated with granules, is present in all cell types.

48. Effect of total body irradiation on the blood corpuscles of adult hypophysectomized rats. Edward C. PLISKE, John F. KENT*, J. G. VAN DYKE* and F. H. BETHELL*, Department of Anatomy and Atomic Energy Commission: Biological Effects of Irradiation Laboratory, University of Michigan.

The effect of irradiation with X-ray (500 r) on the blood corpuscles of hypophysectomized and non-hypophysectomized rats was compared. Irradiation was carried out 21 days after hypophysectomy. Blood samples were taken for study one day prior to irradiation and weekly thereafter for 11 weeks. Irradiation reduced the mean erythrocyte count in non-hypophysectomized rats to a low of 5.9 million/mm^3 at two weeks and to 4.3/mm^3 in hypophysectomized rats at 4 weeks. Recovery occurred in each case but the count failed to reach the level of non-irradiated hypophysectomized and non-hypophysectomized controls, respectively. Hemoglobin concentration paralleled the changes which occurred in the erythrocyte count. The mean hemoglobin of irradiated non-hypophysectomized rats was reduced from 16.1 gm/100 ml to 12.3 at two weeks and in hypophysectomized rats, from 15.9 to 8.5 at three weeks. The hematocrit in hypophysectomized rats was lower than for non-operated rats but regained the level shown by the hypophysectomized controls. Reticulocytes were depressed from 1.33% to .35% in non-hypophysectomized rats and from .38% to .16% in hypophysectomized rats. Recovery in the latter reached 5.91% by 4 weeks. Post-irradiation depression in the number of leucocytes was approximately equal in hypophysectomized and non-hypophysectomized rats, both showing good recovery thereafter. It is concluded that after depression induced by total body irradiation the bone marrow of hypophysectomized rats exhibits a significant but limited capacity to recover.

114. The myo-tendon junction in larval forms of Amblystoma punctatum. Keith R. PORTER, The Rockefeller Institute for Medical Research.

In a further investigation of relationships between extracellular fibers and tissue cells, some preliminary observations have been made on the junction between muscle cell and tendon in the caudal myotomes of Amblystoma larvae. Electron micrographs of this material show that each muscle fiber is clothed in a thin sheath of fibrils which in general are oriented lengthwise the cell. This sheath is closely applied to a thin coating of non-fibrous material which covers the plasma membrane externally. Together these constitute the sarcolemma. At the end of the muscle cell the unit fibrils of the sheath come together into bundles to form the tendonous connection with the cells of the adjacent myotome. Unit fibrils entering the tendon also arise from complex narrow recesses in the ends of the myoblasts. In these early embryonic forms, the unit fibrils are very slender ($\sim$ 100 Å in diameter) and, unlike collagen, show no clear-cut evidence of periodicity. They are not continuous with myofibrils or other intracellular fibers but appear to originate from, and have their insertion in the cuticular layer covering the plasma membrane. The myofibrils frequently terminate one or two microns short of the end of the muscle cell and, in such cases, their only connection with the tendon attachments appears to be through longitudinal arrays of the fine fibrillar elements of the sarcoplasmic ground substance.

159. *The definitive response of the dilator pupillae, the retractor of the nictitating membrane and the blood vessels of the pinna of the cat following sympathectomy.*[1] Adrian F. REED, Department of Anatomy, School of Medicine, Tulane University.

The definitive reaction of the dilator pupillae, the retractor of the nictitating membrane and of the blood vessels of the pinna to preganglionic sympathectomy (cervicothoracic ganglion) and ganglionic sympathectomy (superior cervical ganglion) has been observed in 9 cats, over periods ranging from 18 months to 5 years after operation. The reaction present one year after the activity of the experimental muscles has become stabilized postoperatively has been considered to be definitive. Stabilization of activity at a near normal range has been the characteristic reaction of the three groups of experimental muscles following preganglionic sympathectomy. The definitive reaction of each group of muscles following this type of preganglionic sympathectomy has been characterized by a slight degree of hyperactivity. The definitive reaction of each group of experimental muscles following ganglionic sympathectomy has been characterized by moderate to pronounced degrees of hypoactivity.

The recovery of function has not been parallel in the groups of experimental muscles following preganglionic sympathectomy. Typically recovery of function was most rapid in the smooth muscles of the vessels and slowest in the dilator pupillae.

[1] This investigation was supported by a research grant [PHS B-496 (C5)] from The National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service.

160. *The origin of a familial character: A study in the evolutionary anatomy of moles.* Charles A. REED, College of Pharmacy, University of Illinois.

Anatomists have generally ignored the search for those incipient pre-adaptive changes which, having once become established in a population, can then furnish the basis for a new trend in adaptive evolution, leading ultimately to extreme ecological and anatomical specialization. Of the moles (Talpidae) all but one are uniquely specialized for burrowing by possessing two pectoral characters: (1) On the humerus the pectoral ridge has shifted medially to abut against (sometimes to fuse with) the elongated lesser tuberosity, forming a transverse bicipital tunnel for the tendon of origin of the M. biceps brachii. (2) The clavicle has lost its scapula articulation and instead forms a synovial joint with the greater tuberosity of the laterally-held humerus. Neither character is found in any other mammal. In the most primitive talpid (*Uropsilus*, from the s.w. highlands of China) neither of these specialized characters is found; presumably the animal does not burrow. However, the pectoral ridge is shifted distinctly medial toward the lesser tuberosity, and thus the tendon of origin of the long head of the biceps must pass at an angle around the distal end of this displaced pectoral ridge. It is concluded that this slight change in bone-muscle mechanics, and not an original change toward a humero-clavicular joint, was the pre-adaptive factor which furnished the basis for the extreme specializations found in the evolution of the moles.

116. Electron microscopy of the motor end-plate in intercostal muscle of the rat.[1] James F. REGER*, Department of Zoology, State University of Iowa. (Introduced by F. A. Stromsten)

Intercostal muscle of the rat was fixed in 2% osmium tetroxide. Areas containing motor end-plates were isolated, embedded in methacrylate and thinly sectioned for examination with the electron microscope.

Evidence is presented for the following conclusions: The nerve branches of the motor end-plate are epilemmal in position and accompanied at the motor end-plate area by "arborization" nuclei, which are smaller, have a smoother surface and greater electron density than the sole-plate "fundamental," muscle nuclei. At the level of contact of nerve and motor end-plate the sarcolemma splits, one layer continuing sub-neurally and separating the sole-plate protoplasm of the muscle from the branching nerve fibers, while the other is continuous with the nerve sheath. The sub-neural sarcolemma is perpendicularly laminated in certain areas, the laminations projecting into the sole-plate protoplasm. These laminated areas are depressed regions within which the ramifying nerves apparently lie. The sole-plate protoplasm lying beneath the sarcolemma contains mitochondria and "fundamental" nuclei. The latter are often convoluted on the surface facing the motor end-plate and somewhat more oval in shape than muscle nuclei found in other areas. Evidence is also presented for the fact that the Z membranes extend into the sole-plate protoplasm.

[1] Aided by grant from the National Institutes of Health, administered by H. W. Beams.

45. Correlation of diaphragm excursion in normal respiration with body type — a cinefluorographic study. Irving REHMAN and George D. MASON*, Department of Anatomy, University of Southern California and the University of California, Los Angeles.

Fifty normal young men were grouped as follows into three basic body types; 16 in the ectomorph, 28 in the mesomorph and 5 in the endomorph groups. High speed x-ray motion pictures (60 exposures/second) were taken of these subjects in the erect position. This enabled visualization of the continuous movements of the diaphragm and chest wall during a minimum of two complete normal respiratory cycles. Tracings of the films projected to life size were made and the following measurements obtained: (a) diaphragm excursion at the costo-diaphragmatic junction, the cardiac-diaphragmatic junction, and through a plane midway between the two; (b) chest wall, internal movements, i.e., transverse diameter at the costo-diaphragmatic junction. Correlations were made between the body types and these measurements.

The average diaphragmatic movement in the ectomorph is 29 mm. on the left and 27 mm. on the right; in the mesomorph 28 mm. on the left and 25 mm. on the right and in the endomorph 24 mm. on the left and 25 mm. on the right. The average movement irrespective of body type is 27 mm. on the left and 26 mm. on the right. Diaphragmatic excursion during normal respiration was greater in our series than that found by others. Constants for each body type were obtained by dividing the average diaphragmatic movement by the average transverse diameter and multiplied by the surface area within less than 3%.

78. *Development of the malleus and incus.* Shafik F. RICHANY* and Theodore H. BAST, Department of Anatomy, University of Wisconsin, and Barry J. ANSON, Department of Anatomy, Northwestern University Medical School.

Completing their study of the developmental and adult anatomy of the auditory ossicles in man, the authors have traced the steps in the morphogenesis of the malleus and incus and have determined the nature and extent of structural modifications in the long crus of the latter ossicle, which may continue throughout life.

Bone formation begins in the malleus at the 120-mm. stage and in the incus at 117 mm. (earlier, in both instances, than in the stapes).

Ossification spreads from a single center in each ossicle (at the junction of malleolar head and manubrium, and in the area of continuity of incudal body and long crus). Envelopment by periosteal bone, with matching spread of subjacent endochondral bone, is almost complete and adult dimensions attained at the 222-mm. stage, that is, only 9 weeks after the beginning of ossification.

The manubrium belongs in a special category, since it ossifies endochondrally without perichondral bone, and may, throughout life, remain cartilaginous at its tip. The anterior process is likewise exceptional in that it develops independently as a rod of membrane bone, finally fuses with the malleus at the 161-mm. stage.

Meckel's cartilage undergoes deorganization (beginning in the 161-mm. fetus), its tissue giving rise to the anterior suspensory and sphenomandibular ligaments.

79. *Large amounts of testosterone propionate and the development of sexual behavior in the young male guinea pig.*[1] Walter RISS*, Department of Anatomy, University of Kansas. (Introduced by William C. Young)

Differences in sexual behavior appear to be determined in part by genetic factors (J. Comp. and Physiol. Psychol., 1954). New data supporting this hypothesis are reported. The development of sexual behavior was followed during the first 17 weeks in males castrated within 2 days after birth and injected daily with 500 μg testosterone propionate per 100 gm body weight. Seven were from a heterogeneous stock (under the conditions of this experiment, frequent copulators) and 7 from a strain inbred by brother-sister matings since 1906 (infrequent copulators). Performance was tested weekly with estrous females. Seven untreated intact males from each stock served as controls. Each animal was isolated except for a lactating female during the first 25 days.

In descending order the rate of development as determined by the first mounts, intromissions and ejaculations, and by the score, was (1) treated heterogeneous males, (2) intact heterogeneous males, (3) treated inbred males, (4) intact inbred males. It is concluded that whereas large quantities of testosterone propionate accelerated sexual development, the amount of acceleration was limited by the character of the substrate (soma), presumed to have been influenced by genetic variables. We emphasize however that the basis for differences in the substrate is not wholly genetic — contact with other animals is also involved (Valenstein, this program).

[1] Aided by grants from U.S. Public Health Service.

117. Electron microscope observations on a reptilian myoneural junction.[1] J. David ROBERTSON*, Department of Pathology and Oncology, University of Kansas Medical School. (Introduced by Robert E. Stowell)

Preliminary electron microscope observations on a myoneural junction in the chameleon (Anolis carolinensis) will be presented. In thin sections groups of axoplasmic processes measuring about 1–3 microns in diameter are seen in a zone of myofibril-free sarcoplasm just inside the very thin sarcolemma. The penetration of the sarcolemma by the processes will be described. The intra-sarcoplasmic axoplasm is surrounded by a complex membrane of the order of magnitude of 500–600 Å thickness. The membrane consists of thin dense outer and inner layers with a third distinct layer in between. The two outer layers of the membrane form numerous evaginations into sarcoplasm which appear in cross sections as spike-like projections measuring about 0.3–0.6 micron in length and under 0.1 micron in overall thickness. Mitochondria are seen in clusters in the axoplasm and adjacent sarcoplasm. Small nerve fibers whose myelin sheaths exhibit an approximately 100 Å repeat period are seen in adjacent regions outside the sarcolemma.

[1] Supported by a grant from the National Institutes of Health.

75. Some data on the productivity of the seminiferous tubules of the rat.[1] Edward C. ROOSEN-RUNGE, Department of Anatomy, University of Washington.

In whole mounts of seminiferous tubules stained with hemalum, germ cells and Sertoli cells can easily be observed and counted. In 500 Sertoli cells in stage 5 (Leblond) an average of 8.85 spermatozoa were found per cell. Spermatogonia and primary spermatocytes were counted in photographs from areas in stage 8. For each Sertoli cell, 2.70 spermatogonia and 2.36 spermatocytes were counted. The actual numerical relationship between spermatogonia, primary spermatocytes and spermatozoa is not the theoretical one of 1: 1: 4, but 1: 0.88: 3.28. This is also in marked contrast to Rolshoven's findings (Anat. Anz. 91, 1941). It was concluded that nearly 20% of potential sperm cells are normally deleted during stages which comprise 2/3 of the total duration of rat spermatogenesis. More than half of these are probably lost as primary spermatocytes. Degenerating cells can be demonstrated at certain stages in whole mounts.

From the data it is calculated that 115,000 spermatozoa are produced by a tubule of 1 cm length during one "spermatogenic wave." Therefore, 4.35 meters of tubule or more than 5% of the combined length of all tubules will produce 50 million spermatozoa, the average number in one ejaculate of the rat.

In whole mounts Sertoli cells appear as well defined cells and not as a syncytium.

[1] Aided by a grant from the Biological and Medical Research Fund of the State of Washington.

108. The application of the scanning densitometer to the study of the human cerebral cortex. Ralph ROSSEN* and Maria RYZEN*. Hastings State Hospital, Minnesota. (Introduced by Robert Good)

The application of the scanning densitometer to the study of the cerebral cortex is presented. The frontal lobes of three senile brains were examined. The specimens were removed within three hours after death, treated with standard histological procedure, and blocks of tissue were taken from various regions of the frontal lobe. Alternating sections were stained with cresyl violet, gallocyanin at pH of 1.70, and Feulgen reagent.

The slides were scanned with a narrow rectangular beam of light which after being transmitted through the cortical layers falls on a photocell producing a current varying with the density of the tissue. The recording unit of the machine records this current as a continuous line and thus translates density variations to amplitude variations. One is able to obtain profiles representing the distribution of various components such as cells, fibers, nucleic acids, etc., in terms of the cortical depth.

The application of the densitometer for the cell counts in the cerebral cortex is discussed. The Beer-Lambert Law is used for the calculation of cell number at any point of the recorded curve.

The value of this machine for the study of the cytoarchitectonics is evaluated in the light of the various difficulties as well as the amount of information which such a study offers.

70. Further observations on histological evidence of osseous tissue resorption.[1] Elbert B. RUTH, Department of Anatomy, The Johns Hopkins University.

Basophilic amorphous areas are present in the compacta of rat femurs where resorption is effected in ♀ suckling young while maintained on Ca-free diet, but not present in controls. These areas are designated incipient resorptions sites because of intimate relation to resorption spaces and sequence of changes in histological traits. Traditionally such areas are associated with substitution bones, and named "cartilage rests." The following additional evidence is offered in support of the "incipient resorption" concept. (1) Nonexistence in middle third of femoral cortex of rat 6 days old, but extensive distribution throughout cortex at 17 days accompanying evidence of internal cortical reconstruction. (2) Extension of these areas through appositional lamellae to subperiosteal surface of femoral cortex in experimental adults, and during maturation of immature femora. (3) Greater size and irregularity of shape in cortex and metaphyseal trabeculae as compared to bars of interstitial cartilage between degenerating chondrocytes of epiphyseal cartilage. (4) Demonstration of canaliculi within these areas in trabecular and cortical sites. (5) Demonstration of these areas in maturing nonsubstitution (parietal) bone. The "cartilage rest" theory is an abstraction incapable of objective verification.

[1] Supported by a grant from National Institutes of Health, Public Health Service.

87. Does the M-nadi test locate cytochrome oxidase in leucocytes? Dwight L. RYERSON, Department of Zoology, Pomona College.

It is becoming apparent from the work of Gomori ('53), Lillie and Burtner ('53) and others, and repeated in part by the author, that the classical techniques for the identification of the myeloid cells by the oxidase (and peroxidase) reactions do not locate or identify intracellular sites of enzymatic activity in dry smears.

Work of Hoffman et al. ('51) was repeated with clear evidence that only the mitochondria (as identified with janus green) of living blood cells (including nucleated erythrocytes of non-mammalian vertebrates) show a positive M-nadi reaction; specific granules of living granulocytes are negative. It should be emphasized that air-dried cells of smears (either untreated, or subsequently exposed to a variety of chemical agents, applied at buffered pH levels, and with various times of exposure; and at temperatures between 50°C and 132°C both moist and dry) do not give reactions which are commensurate with customary enzyme (oxidase and peroxidase) reactions.

Therefore it is concluded that in living leucocytes the M-nadi positive granules (mitochondria) may show the presence and locations of an enzyme (presumably cytochrome oxidase, demonstrable biochemically from macerated cell-suspensions); but that the sites of action in fixed leucocytes do not correspond with those of the living cells. In fixed granulocytes the specific granules are positive; fixed lymphocytes are entirely negative while living lymphocytes clearly show positive janus-green and M-nadi reactions on their mitochondria.

61. Morphology of corpus striatum in small mammals. Maria RYZEN*, Mental Health Research Laboratory, Hastings State Hospital, Minnesota. (Introduced by O. Larsell)

The morphology of the corpus striatum in several species of the Insectivora and Chiroptera was studied. Serial sections stained with cresyl violet, activated copper protargol, and Golgi technic were examined. The corpus striatum shows the classical subdivision into the paleo-, archi-, and neostriatum. Nucleus basalis and globus pallidus comprise the paleostriatum and are in close association with the scattered cells of the entopeduncular nucleus of the hypothalamus. The archistriatum consists of a well differentiated amygdaloid nuclear complex. The caudate nucleus and the putamen of the neostriatum are only weakly separated by the diffuse fibers of the internal capsule. Rostrally the caudate nucleus is continuous with the nucleus accumbens septi.

Camera lucida drawings demonstrate the cell types found in the Golgi preparations of the bat. No fiber connections between the neostriatum and the cerebral cortex were found. Only few cells located at the rostral boundary between the putamen and the neocortex send their processes to the deep cortical strata. Fiber connections between the neostriatum and the anterior and medial nuclei of the dorsal thalamus are shown.

The interrelation between the volumes of the neostriatum, the anterior and medial thalamic nuclei, and the frontal neocortex is discussed.

148. *Influence of cortisone on the mortality of x-irradiated adrenalectomized mice.*[1] George A. SANTISTEBAN*, Department of Anatomy, Medical College of Virginia, and J. Z. BOWERS*, Department of Radiobiology, University of Utah. (Introduced by Erling S. Hegre and Thomas F. Dougherty)

The differences in mortality between x-irradiated adrenalectomized non hormone-treated CBA mice and those given daily entraperitoneal injections of graded doses of cortisone (.005, .05, .5, 1 mg/day) were compared by making standard statistical tests on final mortality, and on slopes of the cumulative mortality curves as fitted by the method of least squares.

The final and cumulative mortality of mice adrenalectomized after receiving 650 r total body x-irradiation was significantly greater than that of the intact irradiated and adrenalectomized non-irradiated controls. Cortisone replacement therapy failed to fully restore the radio-resistance of adrenalectomized animals. However, statistical analysis of the fitted curves disclosed that the hormone progressively decreased the slopes, increased the mean survival time and delayed the death times (any per cent level) and day of mean death frequency as the dosage was increased. These relationships were statistically significant.

Comparisons of the slopes of the mortality curves suggest that the increased mortality in the experimental animals was associated primarily with an adrenal cortical insufficiency. The presence of the adrenal cortical secretions thus seems to be essential for the resistance to x-irradiation injury. Cortisone, however, may partially restore radio-resistance in the event of an adrenal cortical insufficiency associated with x-irradiation.

[1] Supported by grants from the Atomic Energy Commission and the Committee on Growth of The National Research Council at the University of Utah.

54. *Activation of the rabbit adenohypophysis by intraventricular injections of histamine.*[1] Charles H. SAWYER, Department of Anatomy, University of California at Los Angeles and Long Beach Veteran's Administration Hospital.

Recent studies indicate that neurogenic control of the adenohypophysis involves humoral mediators. Among naturally occurring agents, histamine has been found in the hypothalamus and hypophysis, and injected histamine stimulates ACTH release. The present investigation inquires whether histamine may also activate gonadotrophin release. Ovulation in the estrogen-primed rabbit was the index of pituitary activation, and electroencephalographic records were taken during treatment. While intravenous histamine failed to induce either changes in the EEG or ovulation, injection of similar total dosages (0.25–0.5 mg) into the third ventricle of the brain under local anesthesia induced bradycardia, tachypnea, salivation and opisthotonus as well as increased electrical activity in the preoptic and posterior hypothalamic areas. However, the treatment was followed by ovulation in only 1/7 rabbits. Conversely, under moderate nembutal anesthesia (25 mg/kg), intraventricular histamine induced ovulation in 6/7 rabbits. The mechanism by which nembutal appears to facilitate the reaction is unknown, but the anesthetic suppresses histamine-induced salivation and convulsions, reverses its effects on respiration and heart rate and alters but does not eliminate

histamine-evoked changes in the electrical activity of the brainstem. The results indicate that intraventricular histamine activates the adenohypophysis by stimulating the hypothalamus rather than by serving as a final chemical mediator to the hypophysis.

[1] Aided by ONR Contract NR 113–223.

165. *Granular structures ("inclusions") in human cerebral cortical biopsies from non-psychotic patients.* Melvin SCHADEWALD, Ira J. JACKSON* and Marilyn BEAVERS*, Department of Physiology and Department of Neurosurgery, University of Texas Medical Branch.

Previous reports by Papez, Papez and Bateman as well as from this laboratory, have indicated the abundant presence of unique granules in cortices and other parts of brains of psychotic patients. Variation of methods of fixation, refixation, mordanting, dehydrating, embedding, pre-staining treatment, and of differentiation in 206 separate combinations have resulted in a uniform and reliable technic for demonstration of these granules. A total of 22 neurosurgical biopsies from non-psychotic patients demonstrates the ubiquitous distribution of these granules in the cortex and their very probable pathognomonic insignificance for mental illness. Analysis with 93 × objective and 15 × ocular demonstrates that the intra-neuronal structures are skeins or branched units. The granules or rods previously described are nodal accumulations on these skeins or branches. Fragments of the intra-neuronal structures appear as if they had been extruded from the nerve cell cytoplasm to the interstitium. The granules are smaller in nerve cells, larger in the interstitium, and larger yet in the perivascular areas. Comparison of these data on non-psychotic biopsies with previously reported results on psychotic biopsies from the cortex reveals no essential difference.

51. *The mode of release of neurosecretory material in the posterior pituitary of the dog.*[1] Ernst SCHARRER and Rowen D. FRANDSON*, Departments of Anatomy, University of Colorado School of Medicine and Colorado A. and M. College School of Veterinary Medicine.

In the posterior pituitary, the quantity of hormone present is directly related to the amount of stainable neurosecretory material. Thus, in dehydrated mammals the demand for antidiuretic hormones reflects itself in depletion of the neurosecretory substance; return to water balance is followed by its reaccumulation in the nerve terminals which surround the blood vessels. The question arises: how does the neurosecretory material enter the circulation? In order to provoke acute release of neurosecretory substance, an antidiuretic and stressing agent, nicotine tartrate, was injected subcutaneously into greyhounds (2 to 10 mg per kg body weight in single or repeated doses 1 to 5 hours preceding death by nembutal). The pituitaries were fixed in Zenker-formol and embedded in paraffin. Sections (5 and 7 micra) were stained with Gomori's chrome hematoxylin-phloxin. In one case, considerable amounts of neurosecretory material were seen within the greatly dilated vessels of the neurohypophysis in whose tissue hemorrhagic areas abounded. Evidently the walls of the dilated vessels had become permeable to formed particles, namely neurosecretory granules and red

cells. This observation confirms corresponding findings of Hanström (1952) in the giraffe and Rothballer (1953) in the rat. It is probable, therefore, that under normal conditions the neurosecretory substance also passes through the walls of the vessels in granular form presumably to be dissolved in the blood.

[1] ONR contract No. 6onr-231-VIII.

164. A histological investigation of anaphylactic reactions and contralateral reflex inflammation in the rabbit's eye. John SCHMEDTJE*, Department of Anatomy, St. Louis University School of Medicine. (Introduced by Kermit Christensen)

By aseptically introducing a soluble foreign protein into the vitreous of a rabbit's eye, the tissue of that eye will be made hypersensitive to that protein. If the same protein is given intravenously two weeks later, an acute aseptic inflammatory tissue reaction occurs not only in the uveal tract of the injected eye, but usually also in the contralateral eye.

This elective sensitization of the other eye cannot be explained satisfactorily on a humoral basis, the sensitization dose of protein having been immobilized in the vitreous of the inoculated eye. The aim of this investigation has been to determine whether the inflammatory process in one eye can cause a reflex inflammatory response in the other eye through neural pathways involving non-visual afferent fibers from the first eye and sympathetic fibers to the blood vessels of the second eye.

To do this sympathetic anaphylactic reactions were studied in normal control rabbits. Cervical sympathectomies were carried out in the experimental group. To date, contralateral flare-ups have not occurred in the sympathectomized rabbits.

These results suggest that the unexplained clinical entities of bilateral secondary anaphylactic iridocyclitis, sympathetic anaphylactic iridocyclitis and sympathetic (well named) ophthalmia may be due to sympathetic nerve impulses, and if so, could possibly be avoided by appropriate drug therapy.

144. Effects of ACTH on pregnant monkeys and their offspring. Ida G. SCHMIDT and Richard G. HOFFMAN, Department of Anatomy, College of Medicine, University of Cincinnati, and the Christ Hospital Institute of Medical Research.

Seven pregnant rhesus monkeys were treated with 20 mg. doses of ACTH daily during the last 17 to 67 days of their gestation periods, one non-pregnant female was similarly treated for 133 days, and ten untreated pregnant monkeys served as controls. This treatment produced a marked reduction in circulating eosinophils but had no effect on the numbers or distribution of other formed elements, or on the concentration of sodium and potassium in serum, or the levels of glucose in whole blood.

Three of the seven pregnant treated monkeys aborted, the other four pursued an uneventful course and gave birth to apparently healthy offspring. All mothers and babies were sacrificed within 24 hours after parturition. Maternal spleens

and lymph nodes showed characteristic suppression of activity. These same organs were unaffected in the fetuses but fetal thymus glands showed cortical atrophy.

Maternal adrenals responded typically to ACTH by hypertrophy of fascicular cells and loss of vacuolation in their cytoplasm. Fetal adrenals, on the contrary, were very small, with enlarged glomerular zones and atrophic fascicular zones — changes similar to those produced in adult adrenals by cortisone. This suggested that excessive amounts of cortical steroids from the enlarged maternal adrenals passed into the fetal circulation and inhibited activity of the fetal adrenals.

69. *Regulation of blood flow through the stomach of the rat.* Harold N. SCHNITZLEIN*,[1] and Calvin A. RICHINS, Department of Anatomy, St. Louis University School of Medicine.

The macroscopic blood supply of the rat's stomach is similar to that of man. The blood vessels are innervated by a nerve plexus lying at the myo-adventitial junction. This varies in extent directly with the amount of muscle in the vessel.

A modification of Persson's ('52) freezing dehydration technique was employed for study of blood flow. The abdominal contents were frozen *in situ* with liquid nitrogen. Tissue was removed and dehydrated with alcohol at —40°C. Sections were stained with hematoxylin and eosin or with the Holmes' silver technique for peripheral nerves. Rapid freezing of the tissue inhibited agonal spasm and made possible relatively direct observation of the physiological vascular conditions.

Intravenous injections of ergotoxine or pilocarpine and subdiaphragmatic vagal stimulation tended to cause engorgement of the capillary beds in the gastric mucosa. Sympathetic stimulation resulted in capillary ischemia. The activity of the submucosal arterioles was greatly affected by the experiments and seemed to indicate that gastric blood flow is regulated primarily by changes in these vessels. Direct application of acetylcholine to the gastric musculature caused blanching of the musculature and mucosal ischemia. The contraction of the stomach musculature, therefore, may also be an important factor in regulation of blood flow.

[1] Public Health Service Fellow.

101. *Some effects of histidine deficiency in the rat.*[1] Earl B. SCOTT, Department of Anatomy, School of Medicine, University of South Dakota.

Young male rats were pair-fed a purified diet composed of sucrose, fat, salts, vitamins and crystalline amino acids. The experimental diet omitted the amino acid under investigation.

The total lack of histidine resulted in severe atrophy of the testes and accessory sex glands. The non-functional state of the testicular interstitial cells was evidenced by the atrophic condition of the prostate and seminal vesicles.

The thymus glands of the deficient rats were not demonstrable grossly. The thyroids were smaller than normal but histologically unaltered. The adrenals were smaller than normal and compression of the zona glomerulosa was apparent.

The pituitaries were reduced in size with extensive alteration of the chromophilic cells of the anterior lobe. The acidophils were reduced in size but their number

and distribution compared favorably with the normal. The Gomori basic fuchsin-paraldehyde stain was used to study the thyrotrophic basophils. These cells were not altered in size, number or distribution. The periodic acid-Schiff (PAS) reaction was used to study the gonadotrophic basophils. By this method it was observed that these cells were greatly reduced in number to the point of their being completely absent.

[1] Supported by a grant from the U. S. Public Health Service.

162. Cytochemical observations in the prostate gland of the dog.[1] Arlene SEAMAN*, Department of Anatomy, State University of New York, College of Medicine at New York City. (Introduced by James B. Hamilton)

This investigation deals with the cytochemical localization, identification, and probable metabolic significance of histochemically demonstrable lipid, polysaccharide, and protein components of normal dog prostate gland throughout its secretory activity. Results show that the prostatic secretion is a lipoprotein polysaccharide complex.

The lipids of the acinar epithelium are predominantly neutral fat, which is secreted into the lumen. Phospholipids, ester phosphatides, cholesterol and its esters also occur in the acinar epithelium. Evidence indicates that most of the phospholipids were associated with mitochondria. The acid-haematein procedure (Baker, '46) followed by pyridine extraction and further corollary evidence shows the presence of ester phosphatides, probably lecithin located in the acinar cytoplasm. No lecithin was demonstrated in the acinar lumen. Birefringent lipids, possibly neutral fats, cholesterol and its esters were located in both epithelium and stroma.

The total PAS reactive material in the acinar cells was found to be due to the oxidation of the 1.2 glycol group polysaccharides. A part of the PAS positive material was demonstrated to be acid muco-polysaccharides by the hialysed iron method (Hale, '46; Rinehart, '50). Interference in the PAS reaction also suggests the presence of unsaturated lipids in the phosphatide class. The basophilia and metachromasia of the secretion antecedents and products will be discussed. The following positive protein tests were obtained: Millon, sulphydryl, xanthoproteic.

[1] This work was done in the Department of Anatomy, Emory University, Georgia.

163. The number of occipital segments in human embryos. E. Carl SENSENIG, Medical College and School of Dentistry, University of Alabama.

3 segments. On the material studied it is evident that there are definitely more than 3 segments involved and at least 4 segments are incorporated into the human occipital bone. The contribution of 5 segments cannot be determined but there is some indication of the existence of an additional one on the basis of the number of nerve rootlets. This questionable segment would be the most rostral of the occipital segments and represents de Beer's 5th head segment or the second metotic somite. Thus there may be some support for de Beer's and Reiter's view that 5 segments are involved in the occipital bone. The existence of this most rostral segment cannot be proved by the descriptive approach but requires experimental evidence.

46. Reticular fiber changes in the human pituitary in the different age groups. William M. SHANKLIN and Carmine D'ANGELO*, Department of Histology, American University of Beirut Medical School, Department of Anatomy, Medical College of Virginia, and Ospedale Psichiatrico Provinciale di Roma.

This study is based on a series of 100 human pituitaries varying in age from the newborn to 96 years. Paraffin sections are stained by hematoxylin-eosin, Masson trichrome, and frozen sections by a Hortega silver impregnation method. In the anterior lobe of the newborn there are already numerous thin reticular fibers. These fibers become more numerous and thicker with increase in age. In the neurohypophysis of the newborn reticular fibers are very sparse and are limited to the walls of the few large blood vessels present. By the twentieth year some reticular fibers extend from the blood vessels into the surrounding area. By the fortieth year there is a considerable increase in these fibers, with some fibers spreading out from the main fiber bundles. This process continues and in the older age groups the original bundles of reticular fibers have mostly disappeared, leaving behind a meshwork of widely scattered reticular fibers. At the periphery of the neurohypophysis reticular fibers are continuous with the collagenous fibers of the capsule.

142. Some relationships of adrenals, gonads, and thyroid to lymphatic tissues and to blood and thoracic duct leucocytes. Marvin M. SHREWSBURY, Jr.* and William O. REINHARDT, Department of Anatomy, University of California (Berkeley).

The effects of adrenalectomy, gonadectomy, thyroidectomy, and treatment with thyroxin, separately and combined, were compared in terms of alterations in weights of thymus and lymph nodes, blood and thoracic duct leucocyte counts, and thoracic duct lymph flow in male rats. Gonadectomy resulted in a marked increase in the weight of the thymus, but had slight effect on the weights of lymph nodes. Adrenalectomy resulted in marked increase in weight of all these tissues. Thyroxin administration was demonstrated to exert a hyperplastic effect on lymph nodes, this effect being accentuated by adrenalectomy, whereas thyroxin administration had no effect on the weight of the thymus in the intact animal. Removal of the thyroid prevented the increases in weight of the thymus and lymph nodes noted after castration and/or adrenalectomy. Thyroxin administration markedly in-

creased the volume of thoracic duct lymph flow when combined with removal of the adrenals and gonads. Changes in the weights of the lymphatic tissues were not necessarily directly related to alterations in numbers of blood or thoracic duct leucocytes. The eosinophilic leucocytosis consequent on removal of the adrenal glands was significantly augmented by removal of the gonads and thyroid. The observed findings are discussed in terms of the relative influence of the various endocrine glands on mass of lymphatic tissues and numbers of circulating leucocytes.

62. *An experimental investigation of the strio-nigral relationships in the cat brain.* Donald T. SMITH*, Department of Anatomy, University of Oregon Medical School. (Introduced by William A. Stotler)

The polarity of fiber connections between the corpus striatum and substantia nigra has been investigated in the cat by destruction of nuclear components and study of resulting degeneration. In a series of cats, lesions were made in the caudate nucleus, putamen, globus pallidus, mesencephalic tegmentum, and frontal cortex. After survival from 10 to 21 days, the animals were sacrificed and sections prepared by the intensified protargol and carbothionine techniques.

Caudate lesions produce an extensive primary degeneration of the striofugal fibers passing ventrolaterally through the internal capsule. This degeneration passes into the globus pallidus and entopeduncular nucleus and shows an almost complete destruction of the afferent fibers to these nuclei. The degeneration then passes to the medial portion of the rostral cerebral peduncle, and at successive levels extends dorsad into the reticulata and compacta of the substantia nigra. No degeneration extends caudal to the limits of the nigra.

Lesions of the putamen result in loss of afferent fibers to the pallidum, particularly its lateral portion, and the substantia nigra. Degeneration of nigral afferent following striatal lesions was accompanied by extensive gliosis in the nigra. Lesions of the pallidum produced degeneration of nigral afferents, presumably by interruption of the striatal outflow.

Examination of Nissl preparations of the cells of the substantia nigra shows only minimal cytological changes resulting from either striatal or lateral tegmental lesions.

155. *On vascular patterns in red and white muscles.* R. Dale SMITH and Rubert P. GIOVACCHINI*, Department of Anatomy, The Creighton University.

In the domestic rabbit although the majority of the muscles are white there are a few which are distinctly red, viz. semitendinosus, pectineus, soleus. This phenomenon provides a supply of distinctly red and distinctly white muscles. In the present study comparisons are made between the vascular patterns of these two type of muscles.

Domestic rabbits were exsanguinated with Ringer's solution and the hind quarters were injected via the abdominal aorta with india ink, vinyl acetate, or latex. The individual muscles were fixed in formalin and cleared in methyl salicylate or sectioned.

In the cleared specimens it was observed that the the white thigh muscles generally received two or three small arteries which, after entering the muscle, branched profusely to end finally in capillaries running parallel to the fibers. In the red thigh muscles the supply came from arteries which, before entering the muscle, branched frequently so that these muscles had a larger number of small branches entering them, for example, the semitendinosus has 8–14 branches entering it. The distribution of these vessels after entering the muscle appears to be similar to that of the white muscles. These cleared specimens also revealed areas in which the ink did not get into all of the smaller vessels and hence along the length of the muscle there appears to be regular alternation of open and closed vessels. Since these unopened vessels are directed at right angles to the fibers, any given fasciculus would have areas of injected vessels alternating with uninjected areas.

The ratio between the number of fibers and the number of capillaries was studied in sections. Areas in which all of the capillaries appeared to be filled were selected for counting in these sections. It appears that the red muscles have about three times as many capillaries per muscle fiber as do the white muscles.

95. *Cytoplasmic inclusions and neurosecretion in paravertebral sympathetic ganglionic neurons of vertebrates; a survey.*[1] Stuart W. SMITH, Department of Anatomy, University of Colorado.

Cytoplasmic inclusions in sympathetic neurons first reported by Marinesco (1904) and interpreted by Gaupp (1939) and others to be neurosecretory have previously been thought rare. Regular occurrence of large, spheroidal, fluid droplets of medium density and refractility in perikarya and their "discharge" into axons of sympathetic neurons in *Bufo marinus* (Smith, 1952) led to examination of sympathetic ganglia in 52 additional species. Sympathetic neurons of approximately one-third of the species, including representatives from every vertebrate class, were found to contain either or both of the following after trichrome staining: (1) More commonly occurring spherical to distorted ovoid, 1–10 μ, dense, refractile, fuchsinophil droplets distributed variously in cytoplasm and sometimes, but not always, in axons and dendrites. (2) Less commonly occurring (a) droplets like those described for *B. marinus*, or (b) enormous vacuoles of watery fluid. Subtypes 2a and 2b stain like collagen; 2b has not been found in processes. The inclusions satisfy criteria of neurosecretory material.

The situation described parallels the status of hypothalamic neurosecretion before Bargmann introduced application of chrome alum-hematoxylin, which demonstrates hypothalamic neurosecretory material in all vertebrates examined. It appears possible that the material of sympathetic ganglionic secretory inclusions is similarly ubiquitous but not everywhere demonstrable by non-selective trichrome methods.

The function of sympathetic ganglionic neurosecretory material is unknown.

[1] Supported by Continuing Research Fund, University of Colorado, and U. S. Public Health Service.

27. *The origin and development of the tail in the frog, Rana pipiens.* Morris SMITHBERG*, Roscoe B. Jackson Memorial Laboratory. (Introduced by C. C. Little)

The origin of the tail primordia in the early neurula (stage 13–14, Shumway) of *Rana pipiens* was localized with the aid of vital staining techniques. Presumptive epidermis, mesenchyme, somites, neural tube and notochord of the tail were mapped out. In addition to the tail structures, the trunk somites and pronephros primordia were localized.

A distinct difference was established between urodeles and anurans as regards the origin of the tail somites and ventral caudal fin. In particular, the presumptive tail somites of the frog are derived mostly from large masses of sub-epidermal tissue lateral to the slit-shaped blastopore. This is in contrast to the posterior neural plate origin of the tail somites of urodeles. The mesenchyme of the ventral caudal fin in the frog is not derived from neural fold tissue but rather from sub-epidermal tissue lateral and posterior to the slit-shaped blastopore. Extirpation and autoplastic transplantations of the tail primordia revealed a lack of regulative potency of the presumptive tail somite tissue and presumptive notochord tissue. Rana presumptive tail somite tissue, as is the case with presumptive trunk somite in urodeles, did not differentiate into muscle tissue when autoplastically transplanted under the ventral epidermis.

10. *Electro-anatomical studies on a projection from the cerebellar paraflocculus to the cerebral cortex.* Ray S. SNIDER and M. M. RUWALDT*, Department of Anatomy, Northwestern University Medical School.[1]

In order to obtain information on the possible functional significance of the paraflocculus, it was desirable to map its projection to the cerebral cortex.

Electrical stimulation of the ventral paraflocculus with threshold stimuli induced major responses in the following cerebral gyri: anterior ectosylvian, middle ectosylvian, sylvian and coronal. Shortest latency responses were located in middle and anterior ectosylvian gyri (latency to peak: 6 msec.). Longer latency responses (16, 20, 30 msec. to peak) were also recorded from anterior and middle ectosylvian gyri as well as sylvian and coronal. It is probably significant that this is the same cerebral region to which "vestibular areas" of the cerebellum project and that it overlaps somatic II and anterior auditory I.

The dorsal paraflocculus is being studied for a cerebral projection. Thus far such a projection has not been found and it is emphasized that should one exist it is different from that of the ventral.

Standard electro-anatomical methods were used consisting of single pulse stimuli at threshold levels applied through bipolar electrodes. The evoked responses were recorded by way of an amplifier and cathode ray oscilloscope.

[1] Aided in part by a contract with the Office of Naval Research.

44. Human mandible: relationship between the alveolar border, the mylohyoid ridge, the lingual plate and the third molar tooth. Benjamin SPECTOR, William A. CURBY* and Benjamin TOY*, Department of Anatomy, Tufts College Medical-Dental School.

One hundred seventeen, unselected, Asiatic, adult, dentulous, dry mandibles were studied to determine (1) the percentile range in mm from the alveolar border to the mylohyoid ridge; (2) the percentile range in mm from the alveolar border to the apex of the third molar tooth; (3) the percentile range in mm of the apex of the third molar above and below the mylohyoid ridge; (4) the thickness of the lingual plate of the mesial and distal roots of the third molar tooth. A statistical analysis of this study indicates that a "normal" pattern of correlation can be posited in the thickness of the lingual plate of the third molar tooth. Of the total mandible population, approximately two-thirds show the mesial plate of the distal root to be below 100 thousands of an inch in thickness. On the basis of repeat measurements, the mesial and distal roots of the third molar tooth show definite reproducibility from an unselected sample of mandibles.

96. Effect of hypothalamic lesions on insulin sensitivity in rats. B. N. SPIRTOS*, Department of Anatomy, State University of Iowa. (Introduced by W. R. Ingram)

Rats with electrolytic hypothalamic lesions of moderate size showed greater sensitivity to insulin, as determined by the hypoglycemic response to 0.1 unit/kg, than did their pair-fed controls. Somatotrophin (Armour lot no. R285-128, 1 mg/day for 5 days) normalized the insulin sensitivity of the operated rats. The lesions producing this result did not necessarily affect the appearance of the target organs of the anterior hypophysis. The histological structure of the pituitaries is under examination. Since somatotrophin (dose as above) alleviated the extreme insulin sensitivity of hypophysectomized rats, whereas it had no influence on the less pronounced but nevertheless markedly increased insulin sensitivity of adrenalectomized rats (which was readily corrected by cortisone, 1 mg/day for 5 days), we adopted the working hypothesis that hypothalamic lesions may enhance the hypoglycemic response to insulin by selectively interfering with the secretion of somatotrophin. Further experiments designed to test this hypothesis are in progress.

71. The structure of bony surfaces in contact with tendons. Donald L. STILWELL, Jr.* and D. J. GRAY, Department of Anatomy, Stanford University School of Medicine.

A microscopic study of the bony surfaces underlying 14 different tendons revealed marked differences in structure. Although the periosteum of the majority of these surfaces consisted of dense fibrous tissue, it was composed of fibrocartilage in approximately one-third of them. Hyaline cartilage covered the area of tendinous contact in only one.

The periostea in contact with tendons from similar areas of different specimens were also subject to much variation. They differed especially in thickness and in density, but variations also occurred in the number of cells and vessels present.

The periostea at the margins of contact with tendons were usually thickened, converting the areas of contact into grooves. The crests so formed were composed of fibrocartilage which served as attachments for ligaments, retinacula, or synovial tendon sheaths. The synovial tissue lining the tendon sheaths stopped abruptly at the area of contact between tendon and periosteum where it was replaced by dense fibrous tissue or fibrocartilage.

8. *An experimental study of the origin of the afferent fibers of the inferior olivary nucleus of the cat brain.* William A. STOTLER, Department of Anatomy, University of Oregon Medical School.

Electrolytic lesions were made by means of the stereotaxic instrument in the rostral brain-stem of a series of cats in the following areas: bilateral mesencephalic tegmentum, including the central gray; bilateral mesencephalic tegmentum and central gray, excluding the red nucleus of the right side; the left red nucleus; left nucleus profundus mesencephali; bilateral mesencephalic central gray; left mesencephalic central gray; left globus pallidus; nucleus centrum medianum and adjacent midline thalamic nuclei. After survival periods of from 10 to 21 days, serial sections of the brain-stem were prepared by the intensified protargol and carbo-thionine techniques.

Bilateral lesions of the mesencephalic tegmentum and central gray produced degeneration of afferent terminals of the principal and medial accessory nuclei of the inferior olivary complex of both sides. Bilateral lesions of the mesencephalic tegmentum and central gray, sparing the right red nucleus, resulted in loss of the afferents of the left principal and medial accessory olivary nuclei, with preservation of the terminals on the right inferior olivary complex.

Lesions of the left red nucleus produced degeneration of the afferent terminals of the homolateral principal and medial accessory inferior olivary nuclei. Lesions of rostral brain-stem areas other than the red nucleus did not degenerate afferents of the inferior olive. Rostral brain-stem lesions do not affect the afferents of the dorsal accessory olivary nucleus; these are interrupted by high spinal lesions.

11. *Muscle fibers of the tongue, functional in consonant production.* Leon H. STRONG, Department of Anatomy, The Chicago Medical School.

The combined vectors of the fibers of the transversus linguae and those vertical is linguae arising from the septum and inserted dorsolaterally on the aponeurosis of the dorsum of the tongue, or by certain fibers in front of the septum, crossed through the center of the tongue *from* surface to surface, frame a set of forces which produce a horseshoe shaped elevation of the dorsal surface, coapted to the lingual dental surfaces and to the contiguous palate. This elevation contacts the lingual surfaces of the dental arcade and the lateral third of the con-

tiguous palate. By specific innervation, any part or parts of this elevation may be contracted.

Palatograms, prepared by Muyskens' method (University of Michigan), of the consonants used in English, show the extent of such contact. These contacts, when all massed together, conform to the area of an approximate horseshoe indicated above.

Categories of contact follow: (1) contact parallels arcade completely; (2) contact parallels arcade from molars to incisors; (3) contact parallels arcade completely but posterior contact increases medially; (4) contact from canines or incisors posteriorly to last molar, with medial margin of contacts parallel; (5) contact from incisors to molars, with medial margin bowed medially; (6) palatine contact contiguous with molars only; (7) palatine contact contiguous with molars and incisors only.

66. *The action of Polyvinyl pyrrolidone upon animal and plant cells.* J. SZEPSENWOL, Winship Clinic, Emory University School of Medicine.

Polyvinyl pyrrolidone (PVP) affects growth of embryonic tissue in cultures in vitro. In 1.0–2.0% concentration it inhibits growth producing cytoplasmic and nuclear changes, while in low concentrations (0.001–0.3%) it stimulates growth. Various mitotic abnormalities were also found in cultures containing PVP.

The effect of PVP upon growth of Allium capa was studied. Resting bulbs of yellow globe variety were placed on top of vessels containing 200–250 cc. of fresh water. After 3–5 days bulbs with a fair number of roots were transferred to similar vessels containing various concentrations of PVP. The liquid was changed every day. Measurements of the growing roots were made twice daily.

It was found that 2.0–3.0% of PVP arrests growth of onion roots almost immediately. A 1.0% concentration slows down growth, but does not arrest it completely, and a 0.5% concentration of PVP affects growth similarly only beginning the 3rd–4th day of exposure. On the contrary, lower concentrations of PVP (0.0001–0.1%) have a stimulatory effect; the treated roots reach rapidly a greater length than the controls.

Normal and treated root-tips were fixed, stained according to the Feulgen procedure, and sectioned, or prepared smears for microscopical studies. In addition to changes in the relative number of dividing cells, certain mitotic abnormalities were found in the roots treated with PVP.

82. *Some of the factors influencing differentiation and function of gonadal tissue.*[1] Elsie TABER, Kathryn W. SALLEY*, Henry HOWE* and Janet S. KNIGHT*, Department of Anatomy, The Medical College of South Carolina.

The hypertrophy of the right rudimentary gonad following removal of the left ovary in the fowl offers a unique opportunity for determining factors influencing the differentiation and function of gonadal tissue. In untreated, ovariectomized birds this tissue usually forms a sterile testis-like organ, secreting large amounts of androgen and estrogen.

In ovariectomized birds, severe hypo-adrenalism retarded the development and function of the rudimentary gonad but did not change its structure. The possibility that this retardation was due to general metabolic factors rather than to lack of a specific adrenal hormone is supported by the fact that ovariectomized birds, starved to fit growth curves of hypoadrenal birds showed similar retardation.

Estrogen or androgen treatment of ovariectomized birds inhibited the medullary component of the rudimentary gonad but allowed the cortical component to differentiate into ovarian tissue, containing apparently normal oöcytes. Since cortical tissue differentiated in 38% of birds ovariectomized within three weeks of hatching, and since it is believed that hormone treatment cannot originate a morphological component, it would seem that the presence of cortex in the rudimentary gonad is not as rare as heretofore reported. The fact that oöcytes developed after ovariectomies were performed at 60 days, when, according to Brode, 1928, no primordial germ cells are present in the rudimentary gonad, suggests that definitive ova do not necessarily arise from primordial germ cells.

[1] Supported by Committee for Research in Problems of Sex, National Research Council, and the National Institutes of Health, Public Health Service (RG 2829).

145. Effect of growth hormone preparations on the response of immature female rats to exogenous gonadotrophins. George B. TALBERT, Department of Anatomy, State University of New York, College of Medicine at New York City.

Two of three growth hormone preparations which were tested greatly inhibited the action of rat pituitary gonadotrophin, but not the action of comparable doses of a commercial FSH preparation as indicated by ovarian weight. There was also some inhibition of uterine growth indicating a reduction in the output of ovarian hormones.

The animals employed in this series of experiments were 22 to 25 day-old albino rats. The growth hormone preparations were all obtained from a commercial source.[1] The gonadotrophins were (1) a commercial FSH preparation[2] and (2) a homogenate of pituitary glands from long time castrate rats.

The experimental animals were injected subcutaneously twice daily for 4½ days with a growth hormone preparation and with one of the gonadotrophins. Control animals were injected with one of the gonadotrophins only, with one of the growth hormones only, or were untreated. All animals were autopsied the morning after the last injection at which time the ovaries and uteri were weighed and fixed for histological study.

Possible explanations for the failure of the growth hormone preparations to inhibit the commercial gonadotrophin as they did the rat pituitary gonadotrophin will be discussed.

[1] Courtesy of Dr. Irby Bender of the Armour Laboratories.

112. Polarization and electron microscope study of the nerve axoplasm. Wayne THORNBURG*, Department of Anatomy, University of Washington. (Introduced by Eduardo D. P. de Robertis)

Axons from the fibers of the frog sciatic nerve were extruded from the surrounding sheaths and observed with the polarizing microscope. The naked unfixed axon was positively birefringent with respect to the axis of the fiber. Using a 1/20 wave length compensator, a mean figure of 6.0×10^{-4} for the total birefringence of the fresh axoplasm was obtained. This value was not modified by fixation in neutral formaldehyde. A form birefringence curve showed a minimum at a refractive index of about 1.56. Approximately one half of the total birefringence is intrinsic. These results were correlated with measurements of the number of neuroprotofibrils of the axon by electron microscopy of sections of axons. The computed partial volume of this component based on measurements and filament counts in electron micrographs may be correlated with the values of form birefringence. In the unmyelinated nerve fibers the partial volume of the filament component is higher than in the myelinated nerve fibers. In addition to the neuroprotofibrils of indefinite length and of about 80–90 Å thick, an axon membrane, mitochondria, endoplasmic reticulum and a dense particulate component were found to be present in the axon.

153. Vagal influence on cardiac function in young puppies.[1] R. C. TRUEX, J. C. SCOTT*, M. Q. SMYTHE*, and D. M. LONG*, Divisions of Anatomy and Physiology, Hahnemann Medical College.

This experimental study is based on 112 animals (1–73 days of age). Electrocardiographic data were used to correlate animal heart rate with age, weight, anesthesia and vagal stimulation. Heart rate was related more closely to heart weight than to body weight and age. In older animals nembutal anesthesia blocked some of the vagal influence and the heart rate was increased. At the moment of tying an exposed vagus nerve, either left or right, such mechanical stimulation temporarily slowed the heart rate. When the tied nerve is then cut and the distal stump stimulated electrically, the heart rate showed an even greater decrease in animals of all ages. The right vagus was more excitable than the left, and produced greater cardiac inhibition. Following unilateral and bilateral vagotomy the heart rate approximated but never exceeded the anesthetic (control) rate. Such physiologic results indicate immaturity of the intrinsic cardiac nervous system although the vagi were excitable at all ages. Histologic study of these neural elements is now in progress and may help explain some of the above results.

[1] Supported by Grant H-321C from U. S. Public Health Service.

14. Studies on involuntary movements at rest in the dog. Calvin TURBES*, Department of Anatomy, Indiana University. (Introduced by Richard L. Webb)

In preliminary studies experimental procedures were used to determine which regions of the nervous system participate in involuntary movements at rest involving the flexor muscles of fore and hind limbs of dogs, following encephalomyelitis.

Surgical, physiological and anatomical approaches to the cerebral cortex, mesencephalon, spinal cord and peripheral nerves were used. The information obtained thus far indicates that the mechanism of genesis of these movements is located in the spinal cord.

These movements cease during stimulation of the contralateral motor cortex, cerebral peduncle and pyramid. They cease "permanently" following dorsal and ventral rhizotomy, tenotomy of the involved muscles and fracture with shortening of the involved limb.

80. Factors influencing the effectiveness of testosterone propionate for sexual behavior in the male guinea pig: An interrelationship between experiential and genetic factors.[1] Elliot S. VALENSTEIN*, Department of Anatomy, University of Kansas. (Introduced by Homer B. Latimer)

Fourteen genetically heterogeneous males and 24 males from the highly inbred Strains 2 and 13 were tested for sexual behavior after being raised in two caging situations. Half the males in each stock were kept with their mothers 25 days and with females of the same age until day 73 (social group), the other half were kept alone with their mothers 25 days, then isolated (isolate group). Seven weekly tests were begun day 77.

For Strain 2 males there were significant differences between the social and isolate groups. All of the males in the social group exhibited the complete sexual act, while none of the isolates exhibited intromissions and ejaculations. A similar, but not significant trend in this direction was noted in the heterogeneous and Strain 13 animals.

The importance of contact with other animals for the maturation of sexual behavior is believed to have been demonstrated, but it seems that this factor operated within the limits of a certain genetic framework. After castration the sexual behavior of Strain 2 males dropped to a baseline, but returned to the higher acquired level with hormonal therapy. This appears to indicate that the effectiveness of testosterone propionate on the somatic substrate is influenced by experience. The significance of the results for anatomy, endocrinology and psychology will be discussed.

[1] Aided by grants from U. S. Public Health Service.

81. The thymus as an extrathyroidal source of thyroid hormone. Uptake of radioactive iodine by thymus tissue following thyroidectomy in adult rats.[1] J. R. VAN DYKE, W. C. FOSTER* and A. W. WASE*, Divisions of Anatomy, Physiology and Biochemistry, Hahnemann Medical College.

The concept that thymus tissue may [illegible]

Male rats *thyroidectomized* at 75 days of age were injected intraperitoneally one year later with 5–10 microcuries of I^{131} per 100 grams of body weight. Measurements of thymic radioactivity following the small dosage (Group I) showed average values of 3.33 in controls and 12.93 in thyroidectomized animals. After a larger dosage (Group II), however, values of 13.60 for control and 20.90 for experimental thymus glands were obtained. This index of iodine storage greatly exceeded that for other tissues. Consequently, it is suggested that the thymus is a major source of thyroid hormone following thyroidectomy due to excessive stimulation by thyrotropic hormone.

Correlated *histological observations,* demonstrating "clusters" of thyroid-like vesicles in many specimens, will be presented; together with radioautographs of the epithelial and lymphoid components of this tissue.

[1] Aided by grant no. 1878 from the National Cancer Institute.

175. Duration of stimulation of the hen's hypophysis in progesterone induced ovulation. A. van TIENHOVEN*, Department of Poultry Husbandry, Mississippi State College. (Introduced by Andrew V. Nalbandov)

Everett and Sawyer (1953) demonstrated species differences between the rat and rabbit with respect to the duration of hypophyseal stimulation for ovulation.

An attempt was made to estimate this time relationship in the hen. A total of 84 hens were injected either with progesterone (0.6 mg./kg.) or with progesterone (0.6 mg./kg.) followed at various time intervals by Dibenamine (30 mg./kg.). The linear regression of per cent failure to ovulate, on time interval between progesterone and Dibenamine injection, was significant between the 2.5 and 1.0 per cent level of probability.

In a series of experiments involving 54 hens, all (24) hens failing to ovulate were autopsied; no evidence of internal ovulation was found. However, in 14 cases signs of atresia of ovarian follicles were observed. In an experiment in which Dibenamine was given in conjunction with ovulation inducing pituitary preparations, it was shown that the atresia observed was not due to Dibenamine administration.

By making use of the mentioned linear regression and the distribution of atresia between the different experimental groups, the time of duration of hypophyseal stimulation was calculated. The minimum time thus estimated was 1 hour and 31 minutes ($P = 0.005$). This seems to indicate that in the hen stimulation of the pituitary and release of LH, in progesterone induced ovulation, take place concurrently.

35. Encephalomyelic dysplasia associated with meningomyelocele. M. Robert VAUPEL*, Department of Anatomy, Tulane University. (Introduced by Homer D. Kirgis)

The central nervous system of 5 fetuses and stillborns presenting meningomyelocele was dissected to determine whether the findings might suggest the cause of the common association of hydrocephalus with this condition.

Each specimen showed gross dysplasia of the cerebellum, lower brain stem, and upper spinal cord. The major and constant abnormality consisted of a

tongue-like prolongation of cerebellar or medullary tissue (or both) through the foramen magnum into the spinal canal. The relationships of this malformation to the spinal cord varied with individual cases. The tongue of tissue lay in a trough-like depression on the dorsal surface of the cord, formed by its incomplete posterior closure. The involved portion of the cord was thinned dorsoventrally, but variably so among the several specimens. The spinal cord was displaced caudally in the spinal canal, evidenced by the cephalically directed nerve roots. The cerebellum was uniformly small and flattened in a supero-inferior direction. The vermis was atypical or entirely lacking. Two cases showed failure of union between the cerebellar hemispheres.

The dissections indicate that the predisposition toward hydrocephalus is associated with faults in drainage of the cerebro-spinal fluid from the fourth ventricle. The fourth ventricle may be completely enclosed within the tongue-like projection of tissue, or, as in one case, completely enclosed by dense connective tissue; the roof is invariably distorted.

104. Contacts between dendrites and axons in the white matter of the hemispheres. A. VAZ FERREIRA, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay.

Dendrites which penetrate from the various cortical areas into the white matter of the hemispheres, frequently reaching its deepest part, were consistently found in about 110 young mice and rats by means of Golgi, Cox and Kopsch techniques. They are seen not only at the places where the white matter contacts directly the caudate nucleus, putamen and Ammon's horn, but also where it covers the lateral ventricle. These dendrites cross some axons of the white matter at variable angles; at the level of the crossing axon and dendrite they are, as far as a careful microscopic study can show, in close contact. The crossings are somewhat reminiscent of the cross-synapse (Cajal), however, with a significant difference: the axonic component is formed, not by thin terminal or collateral branches, but frequently by thick (at times even among the thickest ones in the white matter) and probably axons myelinated after the first weeks. These axons either can be traced up to their origin in cortical neurons or to the striatum, or more frequently they become lost after a longitudinal course in the white matter. Similar dendrites penetrate from the striatum. It is suggested that these cross-contacts could be places of "interaction of neighboring fibers" (Marrazzi and Lorente de Nó). Equivalent contacts are found in other parts of the central nervous system.

105. The velar arterial arches. Hal T. WEATHERSBY*, Department of Anatomy, Southwestern Medical School of the University of Texas. (Introduced by Frank Harrison.)

radial. A superficial radial artery connected with the ulnar in 36% of the arches, being the sole connection in 7%. Supplementing the superficial radial were connections with the origin of the arteries to the thumb and index finger, 16%, the princeps pollicis, 6%, and the volaris indicis radialis, 6%. A median artery contributed to 10% of the arches.

The deep arch was formed by the radial and deep branch of the ulnar except in 9% of cases where a penetrating dorsal metacarpal artery was involved. The deep ulnar frequently, 34%, originated more distally than usually described and passed lateral to the hypothenar muscles, not through them. The volaris indicis radialis was not a proper digital vessel in 86% of arches, giving comparatively large branches to the first common digital of the superficial arch and to the index finger.

155. A gross and microscopic analysis of the lumbar visceral rami in man. Richard H. WEBBER*, Department of Anatomy, St. Louis University School of Medicine. (Introduced by W. F. Alexander)

The visceral rami were carefully dissected and mapped in both cadaver and autopsied bodies. They were removed and prepared for histologic examination by staining with osmic acid, the Nonidez technique, the Holmes technique, or Sudan Black B in propylene glycol.

The visceral rami were found to arise from the lumbar sympathetic trunk at all levels with usually a larger number arising from the levels of L-1 and L-2. No cross communications were found between the sympathetic trunks in the lumbar region. The visceral rami terminated in the aortic plexus including the inferior mesenteric ganglion or contributed to the hypogastric nerves which descended into the pelvis. A considerable number of myelinated nerve fibers were found in all of the visceral rami including sizes which fall within the range of preganglionic nerve fibers. Accessory sympathetic ganglia were numerous. Many of the accessory ganglia contained only a few cells; others contained several hundred. By counting the myelinated fibers in the ramus, both proximal and distal to the site of the ganglion, the ratio of preganglionic nerve fibers to ganglion cells was determined to be about 1 to 22.

119. Structure and reconstitutability of beef muscle dehydrated by freeze-drying. Hsi WANG and Earl AUERBACH*, American Meat Institute Foundation, The University of Chicago.

Thin slices of *Biceps femoris* weighing approximately 5 gm. were pre-frozen at —150°, —80°, or —17°C. and subsequently sublimated in a laboratory lyophilizer (H. S. Martin Co.) under 0.3–0.001 mm. mercury vacuum pressure. Portions of these dried samples were embedded in celloidin, sectioned at 10 μ, and stained with hematoxylin and Van Giesen's. The tissues pre-frozen at —150°C were not physically altered except for the presence of tiny vacuoles in the muscle fibers. Those pre-frozen at —80°C. contained irregularly shriveled muscle fibers and much increased perimysial and endomysial spaces. Those pre-frozen at —17°C. contained even greater abundance of interfibral spaces while the muscle fibers were severely though uniformly reduced in diameter. These different morphological

patterns were produced as a result of differences in the rate and loci of freezing determined by each of the respective pre-freezing temperatures used. But despite these differences they all reconstituted very well upon rehydration both structurally and moisturewise. The observed high degree of rehydratability of frozen-dried muscle tissue was attributed to two essential factors inherent in freeze-drying: (a) the muscle fiber protein apparently underwent no denaturation throughout the process; and (b) the emergence of a continuous system of space to make the tissue spongy.

105. Observations on the cytology of nerve cells from scorbutic guinea pigs. Tim WEATHERFORD*, Department of Anatomy, University of Miami School of Medicine. (Introduced by R. T. Hill)

Guinea pigs two months old were fed a vitamin C deficient diet until they displayed signs of extreme scurvy. The animals were then sacrificed and a section of thoracic spinal cord with the attached dorsal root ganglia was removed for histological study. It was found that ascorbic acid, as demonstrated by the acidified silver nitrate method, was absent in the nerve cell bodies. Other changes such as the following were noted. The mitochondria coalesced and formed small clumps. Chromatolysis was noted in many of the nerve cell bodies. A decrease in periodic acid Schiff positive substance was noted. An alteration in the amount and location of both ribonucleic acid and desoxyribonucleic acid was observed. It is significant to note that there were no demonstrable changes in the Golgi apparatus, acid and alkaline phosphatase activity, and sudanophilia from that previously found by us in the autonomic ganglia cells.

20. The early development of the bronchopulmonary segments. L. J. WELLS and E. A. BOYDEN, Department of Anatomy, University of Minnesota.

This account is based upon a graded series of human embryos that had been used by the first co-author in his study of the developing diaphragm. The lungs were exposed by dissection, drawn in situ and sectioned serially. Then the bronchial and vascular trees were reconstructed in wax.

By Horizon XVII, all segments are present and marked by surface lobulations visible in fresh as well as fixed specimens. At this stage the lungs do not occupy the whole of the pleural sacs; therefore the developing bronchi do not compete for thoracic space. The plane of the lungs is almost frontal, since these organs are compressed by the larger ventrally-placed heart. Cephalad, the lungs have not passed the 1st rib; caudad, they have barely reached the 7th rib. Major growth is therefore caudal and ventral.

59. *Some connections of the midline region and centre median nucleus of the thalamus of the cat.* David G. WHITLOCK and Leon H. SCHREINER*, Department of Neurophysiology, Army Medical Service Graduate School, Washington, D.C., and Mayo Foundation. (Assisted by Walle J. H. Nauta)

Fiber degeneration following focal destructions of the medial and paramedian cell groups of the cat thalamus were studied with silver techniques. Following lesions of the midline, fiber degeneration could be traced to adjacent intralaminar nuclei bilaterally, with some spillover to the dorsomedial, ventrobasal, ventroanterior and rostral pole of the reticular nuclei. Extrathalamic fibers, arising from the site of destruction, coursed through the medial angle of the internal capsule to end in the deeper layers of the orbital gyrus, prepiriform cortex and claustrum. An offset of these latter contributions terminated, in part, in the anterior cingulate region while longer fibers joined the cingulate fasciculus, passing caudally over the corpus callosum into the presubiculum and the entorhinal area. Additional fiber degeneration to the gyrus proreus and medial orbitofrontal cortex was tentatively identified with similar degeneration observed following lesions of dorsomedial nucleus.

Degenerating fibers observed following lesions of the centre median nucleus coursed rostralward and ended, in part, in the intralaminar cell groups bilaterally. Other massive connections passed to the ipsilateral ventrobasal nuclei and to the entire rostral half of the reticular complex. Longer fibers of this rostrally directed group entered the internal capsule and terminated in the ipsilateral lentiform nucleus and claustrum.

23. *Nuclear inclusions in the mouse liver.*[1] J. Walter WILSON, Department of Biology, Brown University.

Nuclear inclusions in the mouse liver have been described by many authors. We have found them in normal mice and in mice subjected to various experimental treatments to produce liver injury or hepatomas. Most frequent are one or more eosinophilic spherical bodies with basophilic material associated with the surface. They are apparently derived from nucleoli because intermediate stages occur, but other nucleoli in the same nucleus may remain normal and in a binucleate cell the other nucleus may be normal. They correspond in appearance to Cowdry's type B virus inclusion but our attempts to transmit them to other mice, as Findlay did successfully, by injecting intraperitoneallly homogenized liver which contained large numbers of inclusions, were unsuccessful. They are usually postive with the Schiff reaction and may sometimes contain fat. They are usually homogeneously granular but sometimes vacuolated, in one instance consisting of a cluster of round vacuoles. They occur not only in liver cells but also in hepatomas and hepatoma transplants. The most probable explanation of their appearance is some disturbance of the cell metabolism as others have suggested, e.g., Andrew who found them in senescent mice. Possibly the virus invoked by Findlay produces a similar disturbance in forming the inclusions he reported.

[1] Supported by a grant from the National Cancer Institute, United States Public Health Service.

20. Congenital malformations in the offspring of rats treated during pregnancy with azo dyes.[1] James G. WILSON, Department of Anatomy, University of Cincinnati.

Gillman, Gilbert and Gillman ('48) demonstrated that trypan blue injected before and during pregnancy in the rat caused congenital malformation in the offspring. To extend this study several additional azo compounds have been tested for teratogenic activity by the following standardized procedure. Each substance tested was injected subcutaneously in 1-cc doses of 1% aqueous solution on the 7th, 8th and 9th days of gestation. The mothers were killed on the 20th day of pregnancy and the young removed, weighed, examined for malformations and fixed for clearing or dissection.

The compounds tested in addition to trypan blue were azo blue, Evans blue, Niagara blue 4B, Niagara sky blue 6B, Congo red and chlorazol black. Only three of these produced malformations in significant numbers of offspring: azo blue in 59%, trypan blue in 49%, and Evans blue in 14%. Hydrocephalus was the most frequent defect after treatment with all three dyes, but ocular, vertebral and other brain malformations were also commonly found after azo blue and trypan blue injections. Other visceral and skeletal structures were occasionally affected.

Although all substances tested were basically similar in chemical structure, the dyes that caused maldevelopment differed from those that did not in that the former possessed the diazotized ortho-tolidine grouping. The teratogenic activity, therefore, appears to be associated with this part of the molecule.

[1] Supported by a grant (A 105) from the National Institutes of Health, Public Health Service.

186. Central nervous regeneration in animals in relation to observations in a human subject. W. F. WINDLE, J. L. LITTRELL, J. O. SMART* and W. AGNEW, Research Division, Baxter Laboratories, Inc.

Regeneration or generation of neurons occur in transected lower vertebrate and, perhaps, young mammalian neuraxes. Adult mammalian central regeneration, usually blocked by glial and collagenous scarring, is abortive. However, central axons, finding defects in pia-glial barriers, exhibit impressive regenerative powers (Tower, '33). Inhibition of barrier formation with bacterial pyrogen (Piromen) permitted substantial intraspinal regeneration after upper lumbar cord transection in adult cats. Apparent restitution of some motor function occurred after 3 months.

It is axiomatic that regeneration never occurs in severed human cords. We submit the case of a woman whose gunshot-severed cord was reunited surgically at the Pennsylvania Hospital in 1901. Evidence that motor and sensory functions were partially restored was presented (Stewart and Harte, '02). Low grade cystitis, possibly generating bacterial pyrogens, accompanied the early recovery period. Some functional gains were maintained 4 or 5 years, but were lost by 15 years. Autopsy 24 years after injury revealed dense collagenous scars with neoplastic loss constricting the cord at the suture site, convincing observers that [illegible] of function I recovery had been erroneous, and relegating them to limbo.

Pyrogen-treated spinal cats, exhibiting coordinated locomotor activities, similarly deteriorated in time. Dense collagenous scars constricted the healing cord, inducing degeneration of the regenerated intraspinal neurons. Successful regeneration will depend on inhibition of glial barriers in acute, and prevention of collagenous scarring in chronic, stages.

176. *Hereditary polymelia combined with edema and sex reversal in the toad.*[1] Emil WITSCHI and Chih Ye CHANG*, Department of Zoology, State University of Iowa.

A toad with an accessory small pelvis and legs was bred to a normal female. Of a total of 2660 eggs, 613 died early. Later 23 crippled embryos were preserved, and 266 tadpoles died early. This mortality of one-third indicates that the eggs were overripe. Of 1758 tadpoles that survived, most were edematous, and only 143 normal. Of the edematous group 293 showed polymelia like the paternal parent. The uniform type of this malformation indicates a hereditary nature with about 40% penetrance. However, manifestation seems related, together with the edema, to overripeness of the eggs; for polymelia was found in edematous individuals only, and not among 143 normal tadpoles. If these deductions are accepted, one should expect that the affected parent also originated from an overripe egg. This opens the possibility that it was genetically female, as already suggested by its size and skeletal character (x-ray). A laparotomy revealed gonads of hermaphrodite composition. The conclusion that both parents were of female genetic type leads to expect an unbalanced sex ratio in their offspring. So far only one-tenth of the young were examined. They show twice as many females as males. The bearing of this complex of abnormalities on problems of sex determination and teratogenesis will be taken under consideration.

[1] Supported by a grant from the National Science Foundation.

147. *Influence of cortisone acetate and methyl androstenediol on rodent adrenals.*[1] Richard C. WOLF*, Bureau of Biological Research, Rutgers University. (Introduced by James H. Leathem)

Adrenal cortical atrophy followed the administration of cortisone acetate to immature male rats. The injection of 1.0 mg of cortisone daily for 7 days abolished fascicular and reticular sudanophilia, while the glomerulosa was lipid rich. Methyl androstenediol (MAD) (2.0 mg daily) counteracted the action of cortisone on adrenal weight and lipids, but had no effect when given alone. In contrast, the male hamster failed to exhibit an adrenal weight response to the steroids and sudanophilic lipids, absent in the control animals, were also lacking after steroid hormone treatment. In the male mouse, 1.0 mg of cortisone acetate daily for 7 days either alone or with 2.0 mg of MAD decreased actual and relative adrenal weights. Halving the hormone dosages and extending the injection period to 20 days provided similar results. In the female mouse, the steroids alone or in combination reduced adrenal weight with androgen causing the more marked effect. Cortisone decreased sudanophilia in the outer fasciculata, while MAD reduced lipids in the juxta-medullary region; the combined treatment was similar to that of cortisone. These data emphasize the species differences in response to steroid hormones.

[1] Supported by a grant from E. R. Squibb & Sons.

196. Cardiac and carbohydrate metabolism during hibernation.[1] Marilyn L. ZIMNY*, Department of Anatomy, Stritch School of Medicine, Loyola University. (Introduced by L. V. Domm)

The following values were taken on adult male thirteen-striped ground squirrels *(Citellus tridecemlineatus)*: body weight, heart rate, respiration rate and body temperature. Half the number of animals were placed in a warm room (25°C.) as controls and the other half were placed in a cold room (3–5°C.) as experimentals. Twenty animals were used for the determination of liver, cardiac muscle and skeletal muscle glycogen. Twenty-four animals were used for the determination of cardiac and skeletal muscle phosphate and lactate levels.

During hibernation the above physiological values were recorded for the hibernating experimental and for the non-hibernating control. Both were then sacrificed and tissue taken for biochemical and histochemical glycogen and phosphate studies as well as biochemical lactate studies.

During hibernation phosphates showed the following percentage changes: (1) cardiac muscle, IP 3.6% decrease, APP 72.4% decrease, PC 31.8% increase; and (2) skeletal muscle, IP 23% decrease, APP 55.3% decrease, PC 37.2% increase. Lactate percentage changes were a 42.5% decrease for cardiac muscle and a 41.4% decrease for skeletal muscle. Sixty per cent of the animals showed decreases in glycogen according to the following percentages: cardiac muscle 72.9%, skeletal muscle 79.8% and liver 98.5%. Histochemical and biochemical results showed a good correlation.

[1] Supported by a grant from the Chicago Heart Association issued to Dr. George Finlay Simmons.

166. The phenomenon of increased light transmission in the initial phases of histological staining with methyl green. Sigfrid ZITZLSPERGER, The Daniel Baugh Institute of Anatomy, The Jefferson Medical College.

PAPERS READ BY TITLE

207. Transparent chamber studies in mice on cellular behavior in vivo. Glenn H. ALGIRE, National Cancer Institute.

Recent developments in transparent chamber techniques as applied to mice have resulted in improved resolution of cellular detail. The motion picture illustrates some results of cinephotomicrographic analysis of both slow and rapid cellular events, as they occur within the living animal. Migration, intracytoplasmic movements, and mitosis are shown for various types of normal and neoplastic cells, and comparisons are made with cellular activity under conditions of *in vitro* tissue culture.

209. The cytological effects produced in the pituitary gland of Bufo boreas by 60,000 r of gamma irradiation. Bennet M. ALLEN, Atomic Energy Project, School of Medicine, University of California at Los Angeles.

Recently metamorphosed toads were given 60,000 r of gamma irradiation by a cobalt emitter and the pituitary glands transplanted (before the death of donors) into immature, unirradiated tadpoles. In a parallel series of control transplants from unirradiated donors, the gland remained normal. Lots from both series were fixed at the end of 8, 16, 24 hours and 2, 3, 5, 10, and 20 days, and sectioned.

At 8 hours, heavy destruction had already occurred in the irradiated transplants, especially affecting clusters of smaller cells. The cytoplasm disintegrated first as was clearly shown in larger cells with intact cell membrane and nucleus. Degenerating nuclei shrank, became dense and stained deeply. At 2 days acidophile granules had all disappeared not being seen again. We do not know whether these cells were destroyed. They will be given special study next summer. This was in sharp contrast to the fact that the basophile cells retained their granules and remained intact throughout the experiment as did a considerable proportion of the non-granular cells.

Clearing away of dead cells and consolidation of the gland were completed at 5 days.

210. The loss of sebaceous glands in the skin of thiamine deficient mice. Thomas S. ARGYRIS*,[1] Department of Anatomy, Harvard Medical School. (Introduced by D. W. Fawcett)

Black mice (C 57) whose hair follicles were in the resting (Telogen) or growing (Anagen V) stages of the hair growth cycle, were offered a synthetic thiamine deficient diet. Pair fed and *ad libitum* controls were used. The thiamine deficient mice began to lose weight within 7 days after the initiation of the deficiency regime. These mice were in terminal stages of the deficiency and many deaths occurred by the 22nd to 25th day. Concomitant with the loss of weight there was a progressive atrophy of the skin. In the terminal stages, the epidermis and hair follicles were reduced in size and the sebaceous glands were either degenerated or had completely disappeared. Although the pair fed controls lost weight, and their epidermis and hair follicles were somewhat atrophied, their sebaceous glands remained intact.

Sebaceous glands elaborate a holocrine secretion and they have a high rate of cell replacement as evidenced by numerous mitoses. Since the Krebs cycle is probably the chief source of the energy required for mitotic division and since thiamine is essential for the normal activity of the Krebs cycle, it may be argued that the atrophy of the sebaceous glands in thiamine deficient mice might be due to the disruption of the cycle and a curtailment of the energy supplied for cell replacement.

[1] Post Doctoral Fellow of the United States Public Health Service, National Institutes of Health.

211. The optical activity of collagen fibers and calcification of bone matrix.[1] James S. ARNOLD* and Webster S. S. JEE*, Radiobiology Laboratory and Department of Anatomy, University of Utah, College of Medicine. (*Introduced by Edward I. Hashimoto*)

A polarized light microscopy study was conducted in conjunction with a radioautographic study of bone growth in rabbits and rats using Ca-45. Strip film radioautograms were prepared of undecalcified bone sections from acetone and alkalinized formalin fixed tissue. The bone sections were decalcified in 5% ammonium alum, and hematoxylin stained through the overlying radioautograms. Examination under the polarized light microscope revealed that the collagen fibers immediately beneath the osteoblastic layer were more intensely optically active than elsewhere in the bone. Von Kossa stains of control serial undecalcified sections demonstrated that these areas of increased collagen fiber optical activity were, in fact, osteoid. A basophilic line separated the optically active osteoid from the calcified bone. In these preparations the osteoid was not metachromatic to toluidine blue staining. In radioautograms of bone from a rabbit sacrificed one hour following Ca-45 administration, it was noted that Ca-45 was concentrated at this basophilic line beneath the osteoid. There appears to be a simultaneous decrease in collagen fiber optical activity coincident with the appearance of metachromasia and calcification of bone matrix. It is not clear whether the occurrence of calcification or a change in the physical or chemical state of the mucopolysaccharides is responsible for the decrease in collagen fiber optical activity. It is apparent, however, that both physical and chemical changes take place in bone matrix coincident with calcification and at a substantial distance from the osteoblastic layer.

[1] Supported by AEC Contract AT(11-1)-119 and the U. S. Public Health Service.

emaciated. In tadpoles, although activity was reduced for a short time by dosages of 20,000 r and above, normal activity or hyper excitability was usual. With dosages of 10,000 r and above, tadpoles often swam in tight circles, the direction being reversible. The effects in both toads and tadpoles appeared earlier and in a greater number with higher dosages.

Although the usual individual differences were observed, toads showed greater sensitivity than tadpoles. At 20,000 r gamma irradiation 50% mortality for tadpoles occurred in 22.5 days, for toads in 11 days. At 40,000 r it dropped to 10 days for tadpoles and to 24 hours for toads, while at 50,000 r it was 6.25 days for tadpoles and 0.6 days for toads.

With dosages above 10,000 r there was an accumulation of fluid in the body wall in some tadpoles. These did not die more quickly than others.

X-irradiation produced all effects of gamma irradiation but at a lower dosage.

208. The effect of 10,000 r of whole body x-irradiation on metamorphosis of Bufo tadpoles. Betty Lapsley BACHMAN*, Atomic Energy Project, Medical School, University of California at Los Angeles. (Introduced by Bennet M. Allen)

Ninety-one tadpoles, divided into groups representing three stages in metamorphosis, were given 10,000 r of x-irradiation. Previous experiments had shown (1) that 1,000 r (in Rana catesbiana) completely suppressed cell division in hematopoietic tissues, and (2) that 20,000 r killed 55 out of 60 tadpoles within 48 hours.

Animals were sacrificed on the sixth day after irradiation (metamorphosis complete in controls of most advanced group). Comparisons were made with controls on the basis of trunk length, tail shrinkage, hind-leg growth, presence of forelimbs, presence of poison glands in the skin, shedding of cuticular teeth and cutting plates, tongue growth, and visceral changes.

All changes characteristic of metamorphosis were definitely observed to continue. In irradiated animals the change did not proceed as far as in control animals.

In the youngest group the initial hind-leg average was 6.33 mm — no forelimbs. After 6 days the hind-legs of the irradiated lot averaged 8.55 mm, controls 9.69. Forelegs had emerged in 36% as against 55% of the controls. Tongue length had increased from 0.56 mm to 0.87 mm with 1.17 mm for the controls. Teeth and plates (present in all at beginning) had disappeared in 40% of the irradiated group, 55% of controls.

In two other groups in which the tadpoles were initially more advanced, 10,000 r likewise slowed metamorphosis but did not prevent it.

213. Endocrine effects of hypothalamic lesions.[1] Russell J. BARRNETT and Jean MAYER*, Department of Anatomy, Harvard Medical School and Department of Nutrition, Harvard School of Public Health.

Minute electrolytic lesions were placed bilaterally in the ventro-median nuclei of the hypothalamus of 38 adult female rats. Of these rats, 16 became hyperphagic, displayed a small, constant elevation of body temperature and had a significant

depression of non-fasted blood sugar. All 38 rats displayed continuous vaginal estrous for varying periods.

Twelve of these rats selected randomly had adrenals and thyroids of normal weight and histology. Their ovaries averaged 42.8 mg and contained no corpora lutea but numerous large or atretic follicles. The uteri averaged 505 mg. Their anterior pituitary glands contained normal cell populations but in some, acidophils were degranulated.

In 6 of the hypothalamic rats which were castrated, the pituitary response was the same as in castrated controls. In 8 hypothalamic rats, the thyroid response to propylthiouracil administration was the same as in controls. The thyroid and adrenal responses of 12 hypothalamic rats exposed to cold at 4°C. for 7 days were normal.

The metabolic and ovarian deficiencies of 28 additional hypothalamic rats were uninfluenced by various hypothalamic extracts. Fiber connections could not be demonstrated between the ventro-median nuclei and the anterior pituitary or the infundibulum in 6 normal controls. The deficiencies described may be due to involvement of higher sympathetic centers.

[1] Supported by a grant from the Ella Sachs Plotz Foundation.

213a. Autoradiographic visualization of in vitro intake of Ca^{45} by the epiphyseal plate in the rat and the effect of hyaluronidase hydrolysis.[1] Leonard F. BÉLANGER, Department of Histology and Embryology, School of Medicine, University of Ottawa.

214. A survey of the second, fourth and fifth digits of hands. Homer BLINCOE, Department of Anatomy, New York Medical College.

Of 502 living young adults, 1002 hands were examined as to relative lengths of index, ring and little fingers. The findings are recorded in three groupings for the index and ring fingers. In 22% of the hands these two fingers are of equal length. In 31% of the cases the index is longer than the ring finger. The ring finger is the longer in nearly 47% of the hands. The data on the little finger were placed under two headings. In 457 hands the little finger does not reach the last joint of the ring finger and in 545 cases it extends to that joint or beyond it. The data include sex and right- or left-handedness. Comparison of the data of the first three groups is made with those of the little finger in the individual cases.

215. Study of metaplasia in the uterus of the rat following estrogen treatment.[1] Walter J. BO*, Department of Anatomy, University of Cincinnati and University of North Dakota. (Introduced by Christopher J. Hamre)

Metaplastic changes due to overstimulation with estrogen were studied in the uterus of the rat.

The animals received a single subcutaneous injection of 2.0 mg. of estradiol dipropionate per week. Uteri were studied microscopically in animals treated for the first time on the 7th or the 21st day of age and autopsied from the 3rd to 39th day after the first injection.

A marked difference in response was observed between the animals treated for the first time on the 7th day of age and those treated on the 21st day. Twenty-two of 30 animals treated for the first time on the 7th day developed pronounced metaplastic changes from the 18th to the 39th day after the first injection. Only 2 of 45 rats treated for the first time on the 21st day of age, however, showed metaplastic changes in the same time interval. It was further noted that estrogen-induced uterine metaplasia begins as numerous independent foci and not as an extension of the stratified cervical epithelium as had been suggested by previous authors. The metaplastic changes did not take place within a short time after the first injection, usually requiring about 3 weeks, but once metaplasia began the process was rapid and the entire uterine epithelium was replaced by stratified squamous epithelium within several days.

[1] Supported by a grant from the Montgomery County Society for Cancer Control, Dayton, Ohio, administered by Dr. William B. Atkinson, University of Cincinnati.

216. Histochemical studies on the area postrema, intercolumnar tubercle, and supraoptic crest in the cat.[1] K. R. BRIZZEE, Anatomy Department, University of Utah College of Medicine.

Methyl green-pyronin, buffered thionin, and gallocyanin-chromalum preparations reveal the presence of relatively large amounts of nucleic acids in cytoplasm of the large cells (neurons?) of the area postrema, intercolumnar tubercle and supraoptic crest. Acid phosphatase and lipase activity (Gomori's revised methods) is localized in both the glialoid cells and large cells, and a positive alkaline phos-

phatase reaction was obtained in the connective tissue sheaths of the sinusoidal blood vessels of these structures. Very fine scattered lipid granules (Kay and Whitehead's Sudan IV and Jackson's Sudan Black) were observed extracellularly in area postrema. No cholesterol (Schultz Method), free amino acids (Berg's Ninhydrin test using Schiff's reagent and sodium hypochlorite), or "Gomori-positive" secretory material (Gomori's chrome alum-hematoxylin-phloxin method), as is commonly found in the neuro-hypophysis, were observed in this structure.

[1] Supported by a grant from the U. S. Public Health Service.

217. *Growth of human sarcoma (HS-1) after intraembryonic transplantation in the 2½-day chick.*[1] Elmer D. BUEKER and Sidlee LEEPER*, Department of Anatomy, University of Missouri.

Through the courtesy of Dr. David Karnofsy the Toolan human sarcoma (HS-1) was obtained. This tumor had been transmitted for 12 generations in cortisone treated rats. From 40 transplant experiments in the embryonic chick 20 specimens were obtained which were sacrificed at 6–17 days of total incubation. This material was prepared with De Castro's modification of the Cajal technique and Wenger's hematoxylin.

This tumor grows as a solid mass in the chick embryo. Little or no infiltration occurs around the edge of the mass. Transplants had increased 4 ×–6 × their original size at 6 days' total incubation, 20 ×–30 × at 10 days', and 40 ×–60 × at 14–17 days'. The transplant was relatively free of peripheral nerves. Growth stimulating effects on spinal and sympathetic ganglia which were observed in chick embryos with implanted mouse sarcomas did not occur.

[1] Aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health.

218. *Some effects of lactation on the metabolism of radium in the albino rat.*[1] Dan BUIE*, Frank HOECKER* and Paul G. ROOFE, Departments of Anatomy and Physics, University of Kansas.

219. *Fluid loss through the skin of the rat.*[1] Earl O. BUTCHER, Department of Anatomy, College of Dentistry, New York University.

Fluid loss through the skin from the dorsum of the rat was determined by stretching the skin over diffusion chambers which contained normal salt solution. The chambers were kept inverted at a temperature of 35°C. and at a humidity of 30%.

Fluid loss through the skin of 22-day-old rats averaged 1.302 mg/cm^2 the first hour. The loss at this age is associated with a thin epidermis, a dry and brittle corneum and a skin having a low fluid content and a low O_2 consumption (Butcher, '41, '43). The fluid loss gradually increases with age and on the 29th day of life it averaged 2.359 mg/cm^2. At this age the skin contains more fluid, has a higher O_2 consumption, a thicker epidermis and a less dry corneum.

When linoleic acid is applied to the skin, the penetration, as determined by fluorescence microscopy, in the 22-day-old rat is greater than in the 29-day-old rat. This penetration in the 22-day-old animal alters the skin, allowing as much as 18.237 mg/cm^2 to be lost in the first hour. By the 29th day, the loss during the first hour after acid treatment is 3.239 mg/cm^2. Linoleic acid is thus more damaging when the fluid content and O_2 consumption of the skin are lowest.

[1] Aided by a grant from John H. Breck, Inc.

220. *The origin and distribution of the anterior chorioidal artery.*[1] Malcolm B. CARPENTER and Charles R. NOBACK, Departments of Anatomy and Neurology, College of Physicians and Surgeons, Columbia University.

Recently neurosurgeons have reported amelioration of rigidity and tremor in paralysis agitans after ligation of the anterior chorioidal artery. Information concerning its origin, course, branches and the structures it supplies is necessary to evaluate its effectiveness and the risks involved. Investigation based on 54 hemispheres of 29 brains is here presented. The anterior chorioidal artery originated from the internal carotid in 74% of these brains and from the middle cerebral artery in 13%, the junction of the anterior and middle cerebral arteries in 4%, and from the posterior communicating artery in 8%; it was absent in 2%. In 90% of the hemispheres in which the anterior chorioidal artery originated from the internal carotid, it appeared first lateral to the optic tract and crossed it twice, and no striate branches came off medial to the vessel. The diameter at its origin averaged 1 mm and its length averaged 25 mm. Consistent branches entered the anterior perforated substance, optic tract, cerebral peduncle, lateral geniculate body and the uncus. The vessel entered the substance of the brain lateral to the lateral geniculate body. Anastomoses between anterior and posterior chorioidal arteries occurred either on the surface of the lateral geniculate or via the choriodial plexus, or at both sites, in 20 out of 21 brains in which the latter vessel was dissected.

[1] Supported by Department of Health, Education and Welfare (B386).

221. *Effect of various temperatures on the induction of parthenogenetic development of rabbit eggs to blastocysts.* M. C. CHANG* and G. PINCUS, Worcester Foundation for Experimental Biology.

Unfertilized rabbit eggs recovered 2–3 hrs. after ovulation were suspended in a pyrex tube containing a mixture of an equal volume of rabbit serum and buffered Ringer's solution. These tubes were stored at different temperatures for various lengths of time. The eggs were then transferred into the Fallopian tubes of a recipient doe. The uteri of the recipient does were removed 6 days later and flushed with saline to determine the proportion of eggs developed into blastocysts. When the eggs were treated with a temperature of 45°, 38° or 24°C. for 10 min. to 12 hrs. no parthenogenetic activation was observed. When they were treated at — 5° or — 10°C. for 3 min. or for 1 hr. no parthenogenetic blastocyst was obtained. In contrast, 3%, 19%, 8% or 4% of transferred eggs developed into parthenogenetic blastocysts when stored at 15°, 10°, 5° or 0°C. for 1 day respectively. The recovered parthenogenetic blastocysts were at various stages of degeneration but about 30% of the recovered blastocysts following the treatment at 10°C. for 1 day were spherical and normal in appearance. The number of chromosomes in the parthenogenetic blastocysts is diploid.

222. *Nerve fibers in the area postrema of the rat.* Carmine D. CLEMENTE,[1] Department of Anatomy, University College, London.

At the level of the obex in the floor of the 4th ventricle is situated the area postrema. Its function is unknown, however, it has been claimed that in the human, neurons exist in this area (Cammermeyer, Acta Anat., *2*, 1947), whereas neurons and nerve fibers were said not to exist in the area postrema of cats (King, J. Comp. Neurol., *66*, 1937).

The medullas of a series of Wistar albino rats have been fixed by perfusion with formal-saline, embedded in paraffin and stained with a modification of the Holmes' slide silver technique. Very delicate nerve fiber impregnations have been obtained. The area postrema in these sections contained many neuronal fibers. Fascicles of fine unmyelinated fibers and thicker fibers could be traced between the adjacent bulbar regions and the area postrema. Within the area postrema some fibers were seen to terminate on the walls of blood vessels. The stained fibers were not glial fibrils or impregnated reticulum, however, the origin of the nerve fibers has not, as yet, been determined nor is it certain whether the area postrema in the rat contains cell bodies of a neuronal type. Further cytological studies are in progress and various other species are being investigated.

[1] Bank of America, Giannini Foundation Fellow, Department of Anatomy, University of California at Los Angeles.

mals subjected to various endocrine operations or hormone injections for a period of 3 weeks. The following findings were made: (1) There was a progressive, significant diminution of the counts with ageing. (2) Adrenalectomy significantly increased, cortisone (6 mg./rat/day) significantly diminished, and desoxycorticosterone acetate (3 mg./rat/day) and lipoadrenal extract (10 Units/rat/day) had no effect on the counts. (3) Thyroidectomy had no effect but thyroxin (130 μg./rat/day) significantly diminished the counts. (4) There was no significant sex difference. Orchidectomy, testosterone (2 mg./rat/day) and progesterone (2 mg./rat/day) failed to influence, but stilbestrol (1 mg./rat/day) significantly diminished the counts. (5) Heparin (2 mg./rat/day), histamine (10 μg./rat/day), protamine (10 mg./rat/day), prolactin (25 Units/rat/day) and pituitary growth hormone (2 mg./rat/day) had no quantitative influence on the mast cells but some of these principles exerted qualitative effects.

[1] Supported by the National Research Council of Canada and Ciba Ltd. The lipoadrenal extract was donated by Upjohn Company.

224. Succinic dehydrogenase activity of cardiac muscle.[1] W. Gregory COOPER*, Department of Anatomy, Columbia University. (Introduced by W. M. Copenhaver)

Histochemical localization of succinic dehydrogenase activity in tissue sections and analysis of tissue homogenates showed a significant difference between the enzyme content of the ventricle and that of the atrium, the ratio varying during different stages of development.

Portions of the right ventricle and atria from embryonic, newborn and adult rat hearts were analyzed by methods employing tetrazolium salts to visualize sites and levels of succinic dehydrogenase activity.

Tissue homogenates were analyzed by the method of Perry and Cumming (1952). In all age groups the ventricle was found to have a higher level of succinic dehydrogenase activity than the atrium. The ratio of ventricular to atrial succinic dehydrogenase increased with time: 1.46 ± 0.08 in the embryo, 2.00 ± 0.09 in the newborn and 2.26 ± 0.09 in the adult heart. An increase in both atrial and ventricular succinic dehydrogenase occurs after birth. Subsequent increases in the V/A ratio are due predominantly to an increase in the ventricular concentration of the enzyme.

Histochemical analysis by the technique of Rutenberg et al. (1953) showed the succinic dehydrogenase activity to be greater within the cardiac muscle fibers of the ventricle than in those of the atrium thereby substantiating the findings of the homogenate study.

Similar parallel histochemical-homogenate studies of cardiac glycogen in the rat are now in progress.

[1] Supported in part by grant H-1221 from the National Heart Institute, USPHS.

225. The effect of estrogen and relaxin on intact and transplanted pubic symphyses in mice.[1] E. S. CRELIN*, Department of Anatomy, Yale University School of Medicine. (Introduced by W. U. Gardner)

A graft consisted of the innominate bones and symphysis from an 8-day old mouse. The hosts were young adult castrated males and ovariectomized virgin

females. The hosts of one group each received a graft placed under the skin of the back. In another group a graft was placed anterior to and in contact with the pubic symphysis of each host. Each donor was the same sex and strain as the host. During the latter 20 days of a 90-day graft-bearing period all of the hosts, except a control group, received daily subcutaneous injections of 1 μgm. of estradiol benzoate and 50 G.P. units of relaxin. At autopsy each host had a pregnancy-type symphysial ligament: 3.8 mm. average length in the males and 6.3 mm. average length in the females. All of the grafts persisted, were well-vascularized and fairly normally-shaped. Although there was only a slight increase in over-all size, they attained a mature ossified state and the marrow cavities contained hemopoietic tissue. The symphysis of each graft consisted of a dense plate of cartilage. There was no evidence, gross or histological, that the transplanted innominate bones and symphyses were affected in any manner by the injected hormones.

[1] Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

226. *The effects of estrogen and relaxin on the pubic symphysis and transplanted ribs in mice.*[1] E. S. CRELIN, Department of Anatomy, Yale University School of Medicine. (Introduced by W. U. Gardner)

Ribs from 3-week old mice were grafted to 5-week old castrated males and females. Each donor was the same sex and strain as the host. Each host received two grafts which were carried for 60 days. One was placed under the skin of the back and the other was placed anterior to and in contact with the pubic bones and symphysis. During the last 10 days of the graft-bearing period, one group received daily subcutaneous injections of 2 μgm. of estradiol benzoate and another group received 2 μgm. of estradiol benzoate and 50 G.P. units of relaxin. A third group served as controls. At autopsy the medial ends of the innominate bones in the injected mice were found to be resorbed, and a symphysial ligament produced. In both injected groups the females showed the greatest response. The ligament was the pregnancy-type in the mice receiving relaxin: 2.0 mm. average length in the males and 3.8 mm. average length in the females. All of the rib grafts persisted, grew slightly and were normally-shaped. Their marrow cavities contained hemopoietic tissue. There was no evidence, gross or histological, that the transplanted ribs were affected in any manner by the injected hormones.

[1] Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

227. *An eosinophil small granule component of the Golgi substance.* A. J. DALTON and Marie D. FELIX*, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

the groups of large vacuoles. These lamellae are approximately 210 Å wide and are thought to be one of the loci of cytoplasmic ribose-nucleic acid. The third consists of small granules varying somewhat in size but averaging approximately 400 Å in diameter. They are intimately associated with the lamellae and in some instances show direct continuity with them. These granules reduce osmic acid only after pretreatment with a strong oxidizing agent (Champy fluid) and, en masse, are responsible for the classic osmiophil reticulum of the Golgi substance. Similar granules with a characteristic distribution in the Golgi zone have been identified in exocrine cells of the pancreas, in principal cells of the duodenum, in hepatic cells and in proximal tubule cells of the kidney.

228. Endocrine function in the hepatectomized rat.[1] Savino D'ANGELO, Catherine STEVENS and Bert SHAPIRO*, The Daniel Baugh Institute of Anatomy, Jefferson Medical College.

Preliminary studies have been made on the endocrine system of adult female rats (Wistar descendants) following surgical removal of approximately two-thirds of the liver (technic of Higgins and Anderson: Arch. Path., *12:* 186, 1931). Seventy-five to 90% of liver regeneration was found to occur in such animals by 7–11 days after hepatectomy. At this time interval, thyroid-body weight ratios in hepatectomized animals were not significantly different from those in sham-operated or untreated control animals. Histologic changes in the thyroids of hepatectomized animals were slight, yet suggestive of decreased function. The 24 hour uptake of tracer doses of I^{131} by the thyroid was diminished but thyrotrophic hormone levels in blood were within normal limits (stasis tadpole method). Weights of pituitary, ovary and thymus were not significantly altered. Adrenal hypertrophy occurred in both hepatectomized and sham-operated animals, with greater enlargement found in the former group. Ascorbic acid concentration in the adrenals was unchanged. These preliminary observations reveal that relatively little disturbance in endocrine function is present in hepatectomized rats after appreciable regeneration of residual liver has occurred.

[1] Supported in part by a grant from the National Institutes of Health, PHS.

229. A comparative histochemical and biochemical study of the lipids in the pyloric caecum of Asterias forbesi.[1] Helen Wendler DEANE and Manfred L. KARNOVSKY*, Departments of Anatomy and Biological Chemistry, Harvard Medical School.

Two diverticula from the stomach extend into each arm of the starfish. In cross-section, a diverticulum appears subdivided into two or three tubes, the walls of which are greatly folded. The tall pseudostratified epithelium of the wall rests on a basement membrane, outside of which there is a serosal layer. When frozen sections of diverticulum are stained with sudan black, the epithelial cells in well-fed specimens display numerous lipid droplets except in the most apical zone. All of the droplets are acetone-soluble. The droplets fail to stain by the Schultz cholesterol test, the Baker test for phosphatides, and by the Ashbel-Seligman carbonyl reaction. The background cytoplasm is moderately sudanophilic and displays a true plasmal reaction, but reveals neither cholesterol nor phosphatide.

Chemical analyses of the diverticula show 8–14% lipid, of which about 1% is phosphorus, 5% acetal lipid, 4% sterol and 3% glycerol ether. The phosphorus content of the phosphatide fraction is low, possibly explaining the negative Baker test.

When cell fractions are prepared by a modification of the method of Schneider and Hogeboom ('50), the "free" fat, which is skimmed off the tops of the tubes, is mostly neutral fat containing little acetal, sterol or phosphorus. The three latter components are more concentrated in the "nuclear," "mitochondrial" and "microsomal" fractions.

[1] Supported by an institutional grant from the American Cancer Society.

250. Some varieties of the arteria recurrens striata media (Heubneri) in the human brain. Charles F. DE GARIS, Department of Anatomy, University of Oklahoma School of Medicine.

The usual a. recurrens striata media arises laterally from the a. cerebri anterior at or slightly apical to the level of the a. communicans anterior and passes between the a. cerebri anterior and the region of the trigonum olfactorium to the substantia perforata anterior. The normal artery as described occurs in 38 basal brain records (17 Whites, 21 Negroes); on one or both sides there are 51 variants (28 Whites, 23 Negroes), often associated with anomalous a. communicans anterior or a. cerebri anterior. Doubling of the a. recurrens occurs 33 times as follows: 17 times (10 right, 7 left) with absence of proximal a. cerebri anterior on same side; once bilateral with large a. cerebri anterior media; twice bilateral with retiform a. communicans anterior; 5 times bilateral, 8 times unilateral with associated large arteries normal. In 1 specimen of a. cerebri anterior media from retiform a. communicans anterior the a. recurrens is triple on right, single on left. In 3 specimens it arises from the a. communicans anterior as a short medial caudally directed common trunk. In 8 specimens (3 right, 2 left, 3 both sides) it is replaced by dense spray of slender arteries that enter substantia perforata anterior separately. In 2 specimens (both sides) short spray assembles into normal sized a. recurrens. In 4 specimens artery arises by two or three roots.

251. Observations on the effect of certain hormones on the growth rate of the incisors of the albino rat. L. V. DOMM and Ruth MARZANO*, The Medical School, University of Chicago and Stritch School of Medicine, Loyola University.

was not disturbed. Cortisone treated females showed the same responses, that is, acceleration in growth, laterality and relative growth rates, as did males.

Hypophysectomized male rats showed marked deceleration in growth. Laterality also occurred. The relative growth rate between uppers and lowers was markedly altered, lowers maintaining a greater growth rate than uppers. Hypophysectomized females gave similar responses, that is, deceleration in growth, laterality, and altered relative growth rates. Cortisone treated hypophysectomized rats showed greatly accelerated rates. In addition, the upper-lower incisor relationship was strikingly altered.

Injections of cortisone in new born rats accelerated the eruption of upper and lower incisors while injections of growth hormone in the dosage used had no, or but a very slight, accelerative effect.

232. Diffusion of esterase after fixation. William L. DOYLE and R. LIEBELT*, Department of Anatomy, University of Chicago.

The prime requisite for valid histochemical staining reactions for enzymes is immobilization of the enzyme. The following data were obtained with sections of rabbit appendix in which the extraction of esterase was measured quantitatively by a micro modification of Gomori's phenyl benzoate diazo method. Comparable pieces of appendix were fixed in acetone, formalin and by freeze drying. Paraffin sections were placed in micro tubes and after removal of the paraffin 25 mm^3 of buffer was added at 25°C. At intervals, aliquots of the supernatant buffer were tested for comparison with the activity residual in the tissue section. Frozen dried material showed the highest activity in hydrolyzing phenyl benzoate and within 10 minutes the enzyme activity was evenly distributed between the supernate and the section. Tissue fixed in cold acetone contained 80 to 90% of the activity of frozen dried material. After 15 to 30 minutes in buffer approximately 90% of the enzyme activity was in the supernatant. Further standing up to 2 hours did not increase the extraction. In such material the non-diffusible 10% could be effective in a truly localized staining reaction. Material fixed one hour in cold formalin had 5 to 8% of the activity of frozen dried material and none of this enzyme was diffusible.

233. Kidney changes related to vitamin E deficiency in rats receiving a diet rich in unsaturated fatty acids. Victor M. EMMEL, Department of Anatomy, University of Rochester School of Medicine and Dentistry.

Weanling rats were placed on an E-deficient diet in which linseed fatty acids (iodine value 172–176) represented 30% of the caloric value. Controls receiving 2.5 mg of vitamin E twice weekly showed no abnormalities after 10 months.

In a group of 32 animals on the E-deficient regime, 9 died during the first 5 weeks, chiefly with respiratory infections. Of the remaining 23 animals, 13 (56%) died or were sacrificed during the 7th to 9th weeks with rapidly developing uremia. Kidneys of these animals were dark purple and firm, and had some glomerular damage, massive proximal tubular damage and scattered peritubular hemorrhages. Bacterial cultures of blood and kidneys generally showed either no growth or occasional contaminants.

Animals surviving beyond the 9th week were sacrificed at intervals up to 24 weeks. The kidneys showed only minor structural abnormalities. However, postmortem autolysis occurred more rapidly in these than in kidneys of control animals. This was also shown in the more rapid rise of NPN in autolyzing kidneys from E-deficient animals, the rate being about 3 times that of controls.

These findings suggest that under the metabolic stress imposed by high intake of long-chain unsaturated fatty acids there is a critical period (7th to 9th weeks) during which lack of vitamin E may lead to renal damage and failure. There is also evidence of chemical alteration in kidneys of animals surviving this period.

234. Internal ear (human). Variant planes of semicircular canals, and concomitant changes in fenestra vestibuli, fenestra cochleae, promontory, bilaterally produced. Thomas Horace EVANS, Department of Anatomy, The New York Medical College and Flower-Fifth Avenue Hospitals.

In specimens having the plane of the superior semicircular canal deflected postero-supero-laterally (Evans, T. H., 1953, Semicircular canal [superior] human ear: three types. Abstract 248, Anatomical Record, 115, no. 2, February) there occurs concomitantly lowered lateral edge of lateral canal (20 to 40 degrees), elevation of rostromedial end of fenestra vestibuli and its plane rotated to face rostrally, tilting of plane of promontory, to overhang superolaterally, fenestra cochleae more medial, and in certain specimens, evidence of brachycephaly. Study of rotation of plane of posterior canal continued. Also, noted relations to plane of malleus, and invasion of mandibular fossa by alisphenoid element (Gregory's epipterygoid). Dissections of both sides and temporal elements in situ.

235. The febrile response of sensitized rabbits to the intravenous injection of antigen. Richard S. FARR, Dan H. CAMPBELL*, Sam L. CLARK, Jr.* and James E. PROFFITT*, Naval Medical Research Institute, Bethesda, and The California Institute of Technology.

sponse to antigen. The similarity of these findings to the febrile response of rabbits to bacterial pyrogens suggests that pyrogen reactions are produced by similar mechanisms of antigen-antibody nature.

236. Effects of corticosterone on blood formation in the adrenalectomized rat.[1]
George J. FRUHMAN* and Albert S. GORDON, Department of Biology, Washington Square College, New York University.

Intact male rats were injected daily for 2 days with 4 mg corticosterone. Adrenalectomy was performed on the 3rd day. Similar injections were continued on this day and for the subsequent 4 days. All rats were killed on the 8th day. Control adrenalectomized rats received 1 ml saline, the vehicle for the corticosterone. Corticosterone prevented the slight drop in RBC count experienced by the saline-treated adrenalectomized controls. It induced marked peripheral eosinopenia but exerted no significant influence upon the peripheral Hb, hematocrit and reticulocyte values. Quantitative evaluation of the absolute numbers of the cellular elements within the bone marrow was made by a previously described method (Fruhman and Gordon, 1953). Calculations indicated that corticosterone had induced, in the adrenalectomized rat, the following changes in the total absolute numbers of marrow cells (millions per femur per 250 gm body wt): an 115% increase in nucleated erythrocytes (63.8 ± 9.4 vs 29.4 ± 4.5), a 41% decrease in neutrophilic granulocytes (48.4 ± 4.9 vs 81.5 ± 8.0) and a 52% decrease in the total numbers of eosinophilic elements (6.1 ± 0.5 vs 12.6 ± 2.6). Femoral marrow weights, total nucleated cellular numbers and lymphoid and mast cell numbers were not altered significantly by corticosterone. This agent caused a 53% decrease in thymic and a 30% decrease in splenic weights. The data suggest that corticosterone acts as an erythropoietic agent in the adrenalectomized rat.

[1] Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

237. Localization and organization of the phrenic nucleus in the spinal cord.
Richard A. GROAT, Section of Anatomy, Mayo Clinic and Mayo Foundation, and Nandkumar H. KESWANI*,[1] Mayo Foundation, University of Minnesota.

Cats were used in this study; the study will be extended to appropriate human material.

Chromatolysis was induced in the cell bodies giving rise to the motor fibers of the phrenic nerve, by sectioning the roots of this nerve; in the cat these roots arise from C-5 and C-6 ventral rami. Serial frontal sections 50 μ thick were stained with thionin. Projections of the sections were traced, with cell groups included. Transverse sections were reconstructed at three levels of the nucleus.

The phrenic nucleus extends through C-5 and C-6 segments, with slight variation among animals. It is a straight, very discrete column of cells lying between

the ventrolateral and ventromedial groups. It exactly parallels the ventral median fissure despite the lateral expansion of the ventral gray column as the cervical enlargement develops.

Thionin, and iron hematoxylin and eosin stains reveal the following: the long axes of the phrenic motoneurones are parallel to the long axis of the cord, a unique orientation, and the cells are decidedly longer than broad; many if not most of the dendrites course longitudinally in the nucleus producing a definite dendritic "tract," another unique feature; there are smaller cells, probably interneurones, both in and adjacent to the nucleus, which seem to be less numerous than in other spinal motor nuclei.

The study will continue with the use of silver and Golgi methods.

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[1] Fellow in Anatomy.

238. Spina bifida and herniation of hindbrain in the offspring of trypan blue injected rats. David L. GUNBERG*, Department of Anatomy, University of California (Berkeley). (Introduced by I. W. Monie)

Fifty-eight female rats of the Long-Evans strain were injected subcutaneously, before and during pregnancy, with 1 ml of 1% aqueous trypan blue, at intervals varying from three to seven days. The total dosage did not exceed 5 ml. Ninety-two of the 373 offspring born were found to be anomalous, seventeen possessing defects which were classified as spina bifida occulta. In every instance, these animals exhibited tail defects, failure of neural arch fusion in the posterior sacral vertebrae, and minor meningeal and cord involvements. Forty-seven offspring possessed defects which were classified as spina bifida aperta, anomalies varying from slight defects to complete absence of vertebral column and spinal cord caudal to the 12th thoracic level. Also, all offspring with spina bifida aperta exhibited a failure of neural arch fusion in at least two vertebrae, accompanied by meningocele, neurorachischisis and neuroepidermal continuity at the site of the lesion. Non-segmented dorsal root ganglia were observed in conjunction with malformed vertebral bodies. An elongation of the skull with herniation of the medulla and posterior lobe of cerebellum through the foramen magnum were observed in 31 of the anomalous offspring. In every instance, this head defect was accompanied by an anomaly of the lumbo-sacral and/or caudal portions of the vertebral column and spinal cord.

239. Further inquiry into the histochemical basis of aldehyde fuchsin staining. N. S. HALMI, Department of Anatomy, State University of Iowa.

Cyanide, after periodate, decreased the AF staining of human gastric chief cells and pancreatic beta granules, but not of elastic fibers and mast cell granules.

Whereas the selectivity of AF for many sulfur containing tissue elements is striking, no valid chemical explanation has as yet been offered for the AF positivity of elastic fibers, gastric chief cells and enterochromaffin (?) granules. The AF staining of elastic fibers cannot be due to adsorbed mucopolysaccharides, since it is as intense in the central parts of thick elastic membranes as it is near their surface.

240. Factors influencing the respiratory pattern of exudate leukocytes. The release of a respiratory stimulating factor from leukocytes.[1] John D. HARTMAN, Department of Anatomy, Temple University.

The in vitro respiratory pattern of exudate leukocytes is characterized by a rapid decrease in respiration rate during the first 20 minutes and then a gradual increase in respiration rate over the next 80 minutes of respiration. The phase of rapid decrease was the object of this study.

Guinea pig exudate leukocytes were suspended in buffered, balanced salt solution and kept at 0° and 37.5°C. At 0, 15, 30, and 60 minutes samples were taken, the cells separated by centrifugation, and the supernates saved. The effect of each sample on respiration was tested by suspending a measured volume of leukocytes in each of the supernates and measuring the oxygen uptake.

A heat stabile factor which increases the oxygen uptake of leukocytes can be demonstrated in the 37.5°C. supernates at 15 and 30 minutes, but not at 0 minutes. This indicates that the factor is initially present within the cells and diffuses out with time. That it is not the result of cell metabolism during the period of standing is indicated by its presence in the 0°C. supernates. It cannot be demonstrated in the 60 minute supernate, indicating its destruction with time or the release of a respiratory inhibiting factor.

The rapid decrease in respiration rate is probably due to diffusion of this factor out of the cells.

[1] Supported by U.S.P.H.S. grant E-525.

241. Electron microscopy of changes in nerve cells following ingestion of lead.[1] J. Francis HARTMANN, Department of Anatomy, University of Minnesota.

A group of 30 rats was given water *ad libitum* containing 10 mg per cent of lead acetate for one month. Sections of tissue fixed in buffered OsO_4 from the cerebral cortex, cerebellar cortex, medulla and spinal cord were studied in the electron microscope. From the 13th day onward, groups of long fibrillar cytoplasmic elements (possibly representing sections through membranes) are present in most cells. They average 120 Å in diameter, are concentrically arranged close to the nuclear membrane, and in some cases nearly encircle it. They are lacking in resolvable axial periodicity or nodosity, and therefore probably do not represent a cytoplasmic counterpart of axone filaments. They occasionally appear as pairs of thin dense lines, strongly resembling the structure of the nuclear membrane. The latter is not detectably altered, and the mitochondria likewise appear normal. The cytoplasm of experimental cells shows increased density, and the endoplasmic

reticulum becomes progressively less prominent. Axones, dendrites and glial elements appear normal, suggesting that the paranuclear fibrillar configurations are the result either of cytoplasmic alteration or of selective deposition of lead in or upon previously electron-transparent structures. Experiments with other metals are planned to aid in deciding between these alternatives.

[1] Supported by a grant (MH-388 from the National Institute of Mental Health, U. S. Public Health Service, and by the Graduate Research Fund of the University of Minnesota.

242. Development of autotransplants of immature adipose tissue of rats.[1] F. X. HAUSBERGER, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

The testicular fat body of the newborn rat consists of a fat free peritoneal fold containing mesenchymal cells indistinguishable at this time from other immature connective tissue cells. Fat deposition begins about seven days after birth. In a group of 6 rats, 4 to 5 days old, one testis was removed together with the fat body, the latter being transplanted subcutaneously in the abdominal wall. Each implant developed into an adipose tissue node of normal histological structure. After 3 months its weight had increased more than 400 times to about 40% of the remaining fat body (average 1000 mg.). In another group (8 rats) the fat body was divided in two equal parts, and in a third group (6 rats) in two parts showing a weight ratio of about 3:1. The pieces, transplanted to symetrical abdominal wall areas, developed into mature adipose tissue nodes showing the same weight ratio as the original donor material. The total weight of the two transplants was again approximately 40% of the remaining fat body. Transplanted neonatal lumbodorsal fascia, although similar to neonatal fat bodies in histological appearance, does not develop into adipose tissue. These experiments show that adipose tissue develops from cells with special potentialities, and that the amount of adipose tissue developed depends, among other factors, on the amount of donor material transplanted.

[1] Supported by Public Health Grant A-215.

ectomized mothers, as was also the volume/body weight; but such differences were not statistically significant (t-test). The other observations likewise indicated a lack of any appreciable difference between the thyroids of the experimental fetuses and those of controls.

We conclude that the hypophysectomy did not induce any significant changes in the morphology of the developing thyroid. The data are not adequate to determine, however, whether physiologically there were any compensatory changes in this fetal gland.

[1] Aided by a grant from the U. S. Public Health Service, RG-2801.

244. The dependence of somitic differentiation on the neural axis.[1] Howard HOLTZER* and Samuel R. DETWILER, Department of Anatomy, School of Medicine, University of Pennsylvania, and College of Physicians and Surgeons, Columbia University.

Strips of 4 successive somites, with or without corresponding neural segments, of Amblystoma embryos (Harrison's stage 25) were grafted into ventral wall or somites of similarly aged hosts. At time of sacrifice two months later grafts of somites without spinal cord could not be identified by either the presence of cartilage or muscle. Grafts of somites with spinal cord in both locations evidenced well formed neural arches and segments of dorsal longitudinal muscle. In another series the neural tube of stage 25 embryos was bisected longitudinally from the 3rd to the 8th segments and a strip of 4 somites introduced between the separated halves. Two months post-operatively each half of neural tube had formed a central core of gray, bounded by a mantle of white matter. The interpolated somites differentiated into a pronounced band of medially running muscle fibers. In addition, each of the twin strands of neural tissue was girdled by its own neural arches. The normal notochord articulated with the parallel set of neural arches.

Previous work indicated that the spinal cord subserved two functions with respect to the neural arches, induction of chondroblasts and ordering their spatial arrangement in anticipation of their definitive architecture (Holtzer and Detwiler, '53). Confirmation of the above and the implication of the spinal cord in the terminal differentiation of myotomic tissues is supplied by these experiments.

[1] Supported by the United States Public Health Service.

245. The treatment of endocrine deficiency in the adult by implantation of fetal glands. I. A preliminary report on the use of the hamster cheek pouch as an intermediary host.[1] E. L. HOUSE, P. F. NACE and G. L. ELLIOTT*, Department of Anatomy, New York Medical College.

Fetal rat pancreas, ranging in age from 17 to 20 days was implanted into the cheek pouch of 110 to 150 gram adult hamsters. Some of the latter were injected with alloxan. Chlortrimeton was given to others. Pancreatectomy was performed on several. Transplanted tissue remained in the pouch up to 7 days. Since this report is based upon 22 cases, the results are more suggestive than conclusive. Within hours, the graft is invaded by mononuclear or polymorphonuclear cells or both, the type having no bearing on the fate of the graft. Most pancreatic

acini deteriorate within 24 hours. Ducts have survived up to 7 days while beta cells were observed after 5 days. Best results were obtained from 17–18 day fetal glands. It appears that Chlortrimeton is of some value. Since 4 or 5 successive subcutaneous or intraperitoneal injections of alloxan at 175 mg./kg. failed to produce anything but a slight, transitory glycosurea, it was concluded that this drug had little effect upon the survival of the implant. The cases of surgical pancreatectomy were too few and variable to be significant. New experiments will concentrate on the use of younger fetal tissue implanted in young, diabetic, desensitized hamsters treated with larger doses of Chlortrimeton.

[1] Supported by the Committee on Research, Council of Pharmacy and Chemistry, American Medical Association.

246. Aplasia of the kidney in mice of strain C58. Katharine P. HUMMEL, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Routine autopsy of 2,391 mice of the C58 strain revealed that in 270 or 11% of them the kidney on one side was either completely missing or appreciably reduced in size. The aplasia occurred twice as often on the right as on the left side and more frequently in males than in females. Approximately one-half of the breeding females in the colony had some young with abnormal kidneys, the rest giving birth to normal young only. Aplastic kidneys, however, were found no more frequently in the progeny of parents with aplastic kidneys than in the progeny of parents with normal kidneys. The abnormal condition was not found to be correlated with either litter seriation or age of mother. A similar kidney anomaly is found associated with eye and limb defects in mice homozygous for the myelencephalic bleb gene, my. Crosses between C58 mice and myelencephalic bleb mice have not produced offspring with kidney abnormalities, demonstrating that the two conditions are not expressions of the same gene.

247. Deviations from normal meiosis in oocytes of Triturus viridescens and their significance in the origin of spontaneous polyploidy. Asa A. HUMPHRIES, Jr.*, Department of Anatomy, University of Virginia.[1] (Introduced by Gerhard Fankhauser)

or non-reunion of the two groups, with the subsequent formation of two second meiotic spindles, each with the haploid number of dyads. Fertilization of such eggs would most probably result in triploidy, but, by retention of the polar body (bodies) at the second division, pentaploidy could result.

[1] Work done at Princeton University.

248. Variation in mitotic activity in the rat stomach at intervals after eating. Thomas E. HUNT, Department of Anatomy, Medical College and School of Dentistry, University of Alabama.

Mitotic activity in glands of the rat stomach varies greatly according to the time elapsed after eating and the amount eaten. In normal animals receiving no food for 48 hours the average number of mitoses is less than 1 per linear mm of a section 6 μ thick. Mitoses are somewhat increased 6 to 8 hours after eating sufficient food to fill the stomach (13 to 15 gms), and continue to increase until a maximum of 6.4 ± 0.9 (S.E. for 10 animals) is reached 20 to 24 hours after eating. Thereafter the number declines gradually to a fasting level at 48 hours. The type of food (chow or meat) does not alter results but reduction to half the amount reduces mitoses by more than half. No significant difference in activity was found between 3 and 15 month animals.

The activity varies considerably within a section, peaks of high activity occurring at 3 or 4 mm intervals. This necessitates examination of more than 4 mm of section to establish an average in one animal. About 90% of mitoses occur in surface cells near the neck of the gland; most others occur in mucous neck cells. A higher percentage of dividing mucous neck cells is found after colchicine. This together with other observations leads to the belief that a transition occurs between surface and mucous neck cells.

249. Electroencephalographic and behavioral effects of stimulation of certain points in diencephalon and forebrain in unanesthetized cats.[1] W. R. INGRAM, J. R. KNOTT* and J. G. PIRSCH*, Departments of Anatomy and Psychiatry, State University of Iowa.

Using a variety of stimulus parameters, various points within the brain were stimulated with bipolar implanted electrodes. Cortical and deep potentials were recorded. Certain of the results are briefly summarized as follows. Hypothalamus — activation of EEG, unrest; from some points progressive rage expression and attack. Nu. vent. med. thal. — activation and alerting. Centromedian Nu. — activation, searching, avoidance. Nu. cent. lat. — activation and arrest. Nu. med. dors. — activation, arousal, searching, apprehension. Nu. vent. ant. — activation. Nu. ant. med. — activation. Nn. vent. post.-med. and post.-lat. — evidence of sensory response, evoked cortical potentials without arousal. Nu. lat. ant. — trance-like state. Nu. lat. post. — activation without external alerting. Caudate Nu. — marked activation, searching, fear reactions. Septum pellucidum — arousal, cry, retreat. Amygdala — facial contractions, licking and chewing, changes in deep potentials, sometimes Jacksonian facial seizures with salivation. Hippocampus — marked deep lead afterdischarges with spread to cortex with electrical evidence of seizures but no apparent loss of awareness.

Afterdischarges with seizure signs were derived from stimulation of Nn. cent. lat., med. dors., lat. ant., and septum. Evoked cortical potentials were obtained by stimulation of Nn. vent. ant., ant. med., centromed., vent. post.-med. and post.-lat., and med. dors.

[1] Supported by a grant from the National Institute of Mental Health, U. S. Public Health Service.

250. Distribution of mast cells in the lymphoid organs of the rat. Allan A. KATZBERG*, Department of Anatomy, University of Oklahoma School of Medicine. (Introduced by John F. Lhotka)

Studies were made of toluidine blue stained sections of spleen, thymus, and lymph nodes of mature normal male white rats of the Wistar strain. The spleen appeared devoid of mast cells and at most only two or three were encountered per section of the entire organ. In the thymus the mast cells were all located at the surface of the gland within the substance of the loose connective tissue of the capsule and along lines of the connective tissue where the capsule formed trabeculae between lobules. The mast cells were elongated and filled with granules and were spaced at fairly regular intervals. None were encountered in the cortex or medulla. In contrast to the thymus, the capsule of the lymph node was devoid of mast cells while fairly large aggregates could be found within the cortex and medulla. None were present in the germinal centers. The cells were more rounded and appeared to have less free space between granules than was the case in the thymus. The maximum spreading out of the cytoplasm and granules of mast cells appeared to be in regions where the thymus and lymph nodes had begun to undergo involution.

251. Effects of X-irradiation on mast cells and mucous secretion in Brunner's glands.[1] Margaret A. KELSALL and Edward D. CRABB*, Department of Biology, University of Colorado.

252. A broncho-esophageal fistula in a 7 mm human embryo. Donald L. KIMMEL, Department of Anatomy, Temple University School of Medicine.

A broncho-esophageal fistula was found in a 7 mm C.R. human embryo recovered from a tubal pregnancy. In serial sections, the specimen shows a normal laryngo-tracheal groove, a complete separation of the trachea and rostral esophagus, a blind esophagus, and a fistula extending from the left bronchus to the stomach. From the caudal end of the laryngo-tracheal groove, the esophagus extends caudad for .32 mm and ends blindly, with no indication of either its epithelial lining or the mesenchymal condensation of its wall seen below the blind termination. The trachea has an extent of .50 mm, and bifurcates normally into the right and left bronchi. The fistula arises from the left bronchus .07 mm caudad to the bifurcation of the trachea and joins the stomach.

Other anomalous tendencies in the embryo include distortion of the neural tube, irregular growth of the pharyngeal arches, and a complete inversion of the tail-end of the embryo. From a study of the irregularity of development, the abnormalities of the embryo appear to be caused by mechanical influences: to be the result of pressures exerted upon the embryo because of its abnormal implantation. It is suggested that the specimen presents evidence that tracheo-esophageal anomalies may be caused by tension exerted upon these structures during early development, and that tracheo-esophageal fistulae are not necessarily produced by genetic factors.

253. Effect of desoxycorticosterone acetate on the adrenal cortex of the hamster. Karl M. KNIGGE*, Department of Anatomy, University of Pittsburgh. (Introduced by Davenport Hooker)

Three groups of male hamsters were given daily, subcutaneous injections of 0.5, 1, and 2 mg of desoxycorticosterone acetate (DCA) respectively for 10 days. Control animals received sesame oil. Hormone treatment does not influence the daily food intake or the increase in body weight. The paired adrenal weight of control animals is 19.4 mg; adrenal weights of DCA-treated groups of animals are 16.8, 15.4, and 13.0 mg respectively for the three dosage levels.

The architecture of the zona glomerulosa, size of its constituent cells, cytoplasmic vacuolation, and mitochondria are not altered by DCA. Esterase activity, normally more intense in this zone than in the remainder of the cortex, is unchanged by DCA. Alkaline phosphatase activity is absent from the zona glomerulosa of control and treated animals.

Adrenal atrophy following DCA treatment is due primarily to reduction in size of cells in the zona fasciculata and zona reticularis. The average cross-sectional area of nuclei of fasciculata cells, as determined by measurement of nuclear diameters, is smaller than normal in animals receiving 1 and 2 mg of DCA. Despite this atrophy, mitochondria of fasciculata cells are not altered significantly by DCA. Esterase activity is unchanged; alkaline phosphatase activity is abolished in the outer portion of the zona fasciculata by 1 mg of DCA and is reduced markedly in the remainder of the cortex by 2 mg.

254. *Experimental control of the size of mesencephalic V nucleus cells in Rana pipiens tadpoles.*[1] Jerry J. KOLLROS, Department of Zoology, State University of Iowa.

Previous reports (Kollros et al., '50; Kollros and Pepernik, '52) indicated that in the larval Rana pipiens the cells of the mesencephalic nucleus of the trigeminal nerve grow rapidly under the direct (and perhaps indirect) influence of thyroxin and that their size maintenance depends upon a continuous supply of the thyroid hormone. Prolonged immersion of Rana pipiens tadpoles in 0.06% thiourea just prior to the period of fore limb emergence produced metamorphic stasis and marked reduction in the size of the big mesencephalic V nucleus cells, in 6 of 8 cases. This tends to confirm that the size maintenance of these cells depends upon a continuous supply of thyroid hormone.

Immersion of 12 hypophysectomized tadpoles into weak solutions of thyroxin (1: 2,000,000,000 or less) for periods of 50–230 days resulted in the development of external characteristics which mark the onset of metamorphic climax. Such changes normally are accompanied by marked growth of the cells of the mesencephalic V nucleus. In none of the 12 animals, however, was any cell growth noted in the mesencephalic V nucleus. The results suggest that these large intracentral neurons require higher thyroxin concentrations for their growth than do cells of the skin glands, mouth parts, legs, jaws, skin windows, etc.

[1] This investigation was supported by research grants (1409 and B-398) from the National Institutes of Health, Public Health Service.

255. *The decidual reaction in the rat after the removal of the antimesometrium.* Robert H. KREHBIEL, Department of Anatomy, College of Medicine, University of Illinois.

256. *A rapid method for the preparation of thin sections of calcified bone and teeth.* B. N. KROPP, Department of Histology and Embryology, Queen's University.

A specimen of thoroughly dried bone, preferably about 2 mm thick, is ground smooth on one side. It is then placed ground side up in a 1" mold assembly of a metallurgical mount press. The specimen is covered by 5 ml. of a powdered acrylic resin ("transoptic"), the assembly placed in the press, and the pressure raised to 100 pounds while the temperature is raised to 130°C. When this temperature is reached, the pressure is raised to 3500 pounds and maintained at that level while the mold cools to 68°C. The pressure may then be released and the 5 mm thick plastic disc, containing the embedded specimen, is expressed from the mold. The specimen is then dry ground on no. 340 grit silicon carbide paper. Fine grinding, also dry, is done on no. 600 silicon carbide paper and continued until histological details appear. The total time required for embedding and grinding is 45 to 60 minutes. Teeth, or thicker specimens of bone, may be prepared in the same way, provided the specimen is thoroughly desiccated prior to embedding. If desired the finished specimen may be mounted on a 1 × 3 plexiglas slide.

257. *The supraoptic crest in the human brain.* Hartwig KUHLENBECK, Department of Anatomy, Woman's Medical College of Pennsylvania.

The diencephalic portion of the lamina terminalis in the human brain retains some characteristics of the subependymal cell plate and also shows certain structural similarities to Henle's so-called velum medullare inferius (pars rostralis taeniae medullae oblongatae, Hochstetter), and to thin parts of ponticulus medullae oblongatae.

Within the basal, thinner portion of lamina terminalis, pars diencephalica, a slight thickening is found in the midline and represents the supraoptic crest of the human brain. This structure consists of an outer zone relatively rich in cells, located beneath the pia, and of an inner zone with few nuclei but a dense texture of parallel spongioblastic fibers running at a right angle to each other, in basodorsal and in transverse direction. The ependymal lining consists of a single layer of very flat, mesothelial-like cells. This thin ependyma becomes again high cuboidal upon approaching the upper third of lamina terminalis praeoptica as well as toward the optic chiasma and lateral parts of preoptic recess.

The cells of the subpial zone resemble those of subfornical organ but there is a tendency to form clusters and occasionally glomerulus-like structures around whorls of aggregated spongioblastic fibers. Sinus-like capillaries are present and a few neuronal elements may occur. The supraoptic crest fits into the group of peculiar para-ependymal structures which also include area postrema and subfornical organ.

258. *Some observations on the role of the aldehyde radical in neurofibrillar argyrophilia.*[1] John F. LHOTKA, Department of Anatomy, The University of Oklahoma School of Medicine.

In an effort to further investigate the role of the aldehyde radical in neurofibrillar argyrophilia (see Stain Techn., *25*, 129; *28*, 101, 129) blocking experiments with *p*-nitrophenylhydrazine, 2–4 dinitrophenylhydrazine, dimethyldihydroresorcinol, and hydroxylamine were performed using the Cajal block and the Protargol slide silver methods. Blocks and sections of appropriately fixed cerebellum and cerebrum were treated prior to silvering with these reagents under varying conditions of time, temperature and concentration followed by thorough washing to remove all traces of the reagents. They were then processed according to technic. Microscopical comparison of the "blocked" sections with "unblocked" control specimens revealed no discernible difference in neurofibrillar staining, strongly indicating that the simple aldehyde radical such as might be blocked by these reagents plays little or no part in the neurofibrillar argyrophilic phenomenon.

[1] Supported by a grant from the Biological Stain Commission.

259. *Effects of vitamin A deficiency on skin and hair growth in C57 black mice.* Lois A. LOEWENTHAL*, Department of Biology, Brown University. (Introduced by William Montagna)

C57 black mice, two months of age, were placed on a vitamin A-free diet. They were bred and their litters were pair-fed. In each pair, one was fed the vitamin A-free diet; the other, of the same sex and age, received the same diet supplemented with vitamin A. Every 12 days alternate areas on the back of each animal on the left side were plucked of their club hairs to initiate growth of hair. The right side was clipped. Daily observations were made on the appearance of the skin on the plucked and clipped sides. Spontaneous growth of hair rarely occurred on the clipped sides of deficient animals. Food intake of these animals did not decrease during the experiment. Deficient animals gained weight up to two months. The weight was maintained for another few weeks and then it decreased sharply. Hairs in the plucked regions grew more slowly than in controls. In deficient mice, the hair was 1–2 mm. shorter than that of controls. In some acutely deficient animals plucking of club hairs failed to stimulate regrowth. Pieces of skin excised 21 days after plucking still contained growing hairs, whereas in control animals, hair follicles were in the resting stage. The epidermis, though thinner, was normal. The distribution of –SH groups, glycogen and metachromatic substances was normal.

In relation to age, the femurs of combined sexes increased in mean length from 32 to 88 mm; femur diameters, from 3 to 7 mm; ilium lengths, from 13 to 49 mm; lengths of os coxa, from 24 to 92 mm; interacetabular widths, from 9 to 30 mm; maximum widths of pelvis, from 19 to 59 mm; and intertuberal widths, from 8 to 25 mm.

The primary data were regrouped in relation to body weight and the same statistical constants were determined. Empirical formulae based on mean values were developed to describe trends of increase in relation both to age and body weight.

In relation to age, the femur of the male tended to be slightly longer, whereas maximum pelvic width tended to be somewhat less than that of the female. However, interacetabular width of the female averaged approximately 9% greater than that of the male. Less consistent differences between male and female values were observed in other categories of the time series.

[1] Aided by the University of Missouri Research Council.

261. Effect of thyroxine on the histologic structure of the mouse submaxillary gland. Cyril LUCKMAN* and Arthur KIRSCHBAUM, Department of Anatomy, College of Medicine, University of Illinois.

The mouse submaxillary gland is sexually bimorphic. The acini of the female are mucoid with few exceptions, whereas in the male a large number possess eosinophilic secretion granules. The female submaxillary gland is converted to the male type by the administration of androgenic hormone. Following castration the masculine gland becomes feminine. In the rat the effect of androgen on the submaxillary gland is dependent upon thyroid function (Grad and Leblond, '49).

Adult male DBA/2 mice were castrated and were thyroidectomized one week later by administering radioiodine. Eight weeks after giving I^{131} (9 weeks postcastration) the submaxillary gland had become degranulated. Eosinophilic acinar granulation was maintained after radio-thyroidectomy if the castrated males received either 500 micrograms of testosterone propionate weekly in oil, or 6 micrograms of thyroxine daily. The submaxillary glands of either intact, ovariectomized, or ovariectomized-adrenalectomized females became heavily granulated if they received 25 micrograms of thyroxine daily.

It appears that either androgenic hormone or thyroxine can independently maintain or induce eosinophilic acinar granulation in the mouse submaxillary gland. The doses of thyroxine used were large, and the normally functioning thyroid does not produce this effect.

262. The aqueous mucoid fluid of the pulmonary alveolar wall. Charles C. MACKLIN, Department of Histological Research, University of Western Ontario.

Just as the gills are washed by an aqueous solution from which their capillaries extract oxygen and into which they give up carbon dioxide, so the alveolar walls of the lungs, the functional descendants of the gills, are washed by an aqueous mucoid fluid (AMF) which yields oxygen to the blood and receives carbon dioxide therefrom. "Plus ça change, plus c'est la même chose." AMF, sensed mainly by indirect evidence (remnants are visible with the light micro-

scope) is produced by pneumonocytes perhaps analogously to glomerular urine by epicytes, but a mucoid element is added probably by granular pneumonocytes. AMF drifts slowly over particle receptors of phagocytic pneumonocytes which relieve it of much of its dust content. Some 400 cc³ per day (human) is lost by evaporation, and most of the rest escapes to lymph capillaries via the outlets. Here more dust particles are removed by RE cells. Tubercle bacilli, thus lodging, incite the tubercle reaction. Cells of alveolar cancer may invade lymph channels at these points. The total volume of AMF in man has been estimated as 20 cc³. It forms a film perhaps 0.2 μ thick on the alveolar wall, contains acid mucopolysaccharides and myelinogens and maintains a constant favorable surface tension. Antibiotic and conservational properties are claimed for it.

265. Sexual differences in epidermal nuclei of human skin. Eve MARBERGER* and Warren O. NELSON, Department of Anatomy, University of Iowa.

Moore, Graham and Barr (Surg. Gyn. and Obstet., 96: 641, 1953) have reported the existence of sexual differences in the chromosomal content of human cells. Sixty-nine percent of the epidermal nuclei in skin biopsies from females contained a mass of chromatin close to the nuclear membrane. In males such masses were seen in five percent of the epidermal cells. A study has been made of skin biopsies from 18 males and 8 females using the technic described by Moore, et al. The males included three cases of hypospadias, one of cryptorchidism and one of hypospadias and cryptorchidism. The females included three cases of pseudohermaphroditism, one having been raised as a boy. The average percentage of nuclei with the chromatin masses in our series of females was 64 with a range of 51 to 77 percent. The three cases of pseudohermaphroditism showed averages of 67, 60 and 71 percent. In the male series the average percentage of nuclei with such chromatin masses was 4 with a range of 2 to 6 percent. The cases of hypospadias and cryptorchidism fell well within the range. It may be noted that abdominal skin appeared to provide better material than skin from other areas studied. Harris hematoxylin afforded somewhat better staining than other procedures attempted.

266. The effect of castration, hypophysectomy, testosterone, and gonadotrophins on the development of the sex accessories and secondary sexual characters in the male Taricha torosa. Malcolm R. MILLER and Marilyn E. ROBBINS*, Department of Anatomy, Stanford University.

sex character development than does a merely moist environment. Testos is moderately effective in stimulating skin and cloacal gland developme the vas deferens, but hypophysectomy inhibits these stimulating effects gonadotrophins of PMS will not produce evacuation of sperm from the testis but are effective in inducing spermatogenesis in the normally quiesce mature testicular lobule at the breeding season. PMS also produces meso lipid deposition, adrenal atrophy, and, in combination with hypophysecto usually lethal. No synergistic effects were observed from the combination o and testosterone. Apparently very specific psychological, nutritional, and logical conditions are required for the normal development and maintena the reproductive state in *Taricha torosa*.

265. *Abnormalities of the urinary system of rat embryos resulting from m pteroylglutamic acid deficiency from days 10–13 of gestation.* I MONIE, Marjorie M. NELSON* and Herbert M. EVANS, Departm Anatomy, and The Institute of Experimental Biology, University o fornia (Berkeley).

Maternal pteroylglutamic acid (PGA) deficiency from days 10–13 of tion results in abnormalities of the urinary system in 66% of fetuses when ined on day-21. Such anomalies are principally variations in kidney size, position and number, hydronephrosis, and hydro-ureter. To determine the opmental disturbances responsible for such abnormalities, rat embryos, a to 21 days, from PGA-deficient mothers, were serially sectioned and co with controls. In the former, the kidneys showed retarded differentiati pecially in the younger embryos, while hydronephrosis and hydro-ureter we tures from day-18 onwards. Incomplete "ascent" of the kidney was fre and, in several fetuses, was associated with abnormality of position of the um artery of the same side. In one day-21 fetus the left kidney was buried dorsal abdominal musculature posterior to the common iliac artery. Separat the ureters from the mesonephric ducts was delayed until day-17 (day-15 i trols). Closure of the caudal portions of ureters was frequent in older f and usually associated with hydronephrosis and hydro-ureter. The caliber urethral lumen was markedly reduced just cranial to the entry of the meson or Müllerian ducts in specimens from days-17 to -21. All the PGA-deficie bryos were smaller in size than the corresponding controls.

266. *Growth of the rat calvaria. The nature of the suture and its role as of growth.*[1] Melvin L. MOSS*, Department of Anatomy, Columbia U sity. (Introduced by Charles R. Noback)

Normal calvarial morphology depends on the activity of the osteogenic t on all surfaces. The shape of the bones is not predetermined by the lo of the sutures. It is possible to produce osseous overgrowth with consequen placement of the sutures by many extirpative technics. This phenomeno

pears to depend on the period of development of the bones, not of the soft tissues. Sutures mark the plane of articulation between adjacent bones. Their location is determined by the relative growth of the bones.

The sutures are not the chief sites of growth. The existence of an expansive force within the sutures during the period of greatest growth is not substantiated. Extirpation of the sutures, singly or severally, is not followed by any decrease in either width or length of the calvaria.

Conflicting theories of calvarial growth are harmonized when allowance is made for the period of development of the site of growth of the particular form under investigation.

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[1] Supported by a grant from the National Institutes of Health.

267. Histogenesis of bone in thallium treated chick embryos.[1] Shirley MOTZKIN*, Gerrit BEVELANDER and Percy L. JOHNSON, Department of Histology, New York University College of Dentistry, and the Graduate School of Arts and Sciences.

In studies of thallium injected chick embryos, Karnofsky et al.[2] have reported marked gross malformations. Our interest in these embryos from a developmental point of view has led us to make a careful histological study of the cartilage replacing and membrane bones. .05–.2 c.c. of a 10 mg./c.c. solution of thallium sulfate was injected into the yolk sac of eight day old chick embryos. Subsequently, the embryos were sacrificed at intervals between nine and eighteen days of total incubation and routinely fixed and sectioned for histological study.

One of the most noticeable effects occurring in thallium treated embryos is the reduction in size of the bones. Microscopic examination of these bones shows an arrested proliferation and delayed resorption of the cells comprising the cartilage model. The persistence of this condition is observed in bones throughout the 12–16 day incubation period. Metaplastic connective tissue associated with the perichondrial region of the long bones, subsequently is transformed into excessive circumferential diaphyseal cartilage. This condition coupled with the presence of modified periosteal bone lamellae results in a marked barrelling effect of the bone. In older embryos, asymmetrical and differential deposition of periosteal bone in conjunction with a probable antagonistic tension of opposing muscles produces marked curvatures. The belated and displaced osteogenic buds appear to be correlated with delayed cartilage resorption, again reflected in latent bone deposition within the shaft. Flat bones of treated embryos, likewise, develop more slowly, are reduced in size and are composed of more compactly arranged lamellae. Increased numbers of osteocytes at these loci appear to be related to diminished ground substance.

Gross and microscopic analysis of the bones in thallium treated embryos shows

268. Sex chromatin in neurons of human frontal cortex and sympathetic ganglia.[1] Margaret MYLLE* and Margaret A. GRAHAM*, Department of Microscopic Anatomy, University of Western Ontario. (Introduced by H. A. L. Skinner)

Nerve cell nuclei were studied in cortical biopsies from 23 female and 7 male psychotic patients, and in similar specimens obtained at autopsy from 12 female and 20 male non-psychotic subjects. In 10 μ sections of female specimens stained with cresyl echt violet, approximately 80% of the nuclei contain a mass of sex chromatin which is usually located against the nuclear membrane. A similar chromatin body is seen rarely in male nuclei.

Nuclei of sympathetic ganglion cells (autopsy specimens from 38 females and 50 males) show a similar morphological difference, according to sex, although the amount of general particulate chromatin makes these cells not quite so suitable for study, compared with some other species. The sex chromatin of female sympathetic neurons is usually located at the nuclear membrane. It varies in shape from a spherical mass, with a characteristic pale central area, to a thin disc, often appearing as a slight thickening of the nuclear membrane. The sex chromatin frequently intervenes between the nuclear membrane and an eccentric nucleolus.

The position of the sex chromatin of these human nerve cells is worthy of note, since it is usually adjacent to the nuclear membrane, rather than the nucleolus as in such species as the cat.

[1] Supported by a grant from the National Research Council and National Health Grants of Canada.

269. Hormonal influences upon the mast cells of peritoneal fluid.[1] Jacques PADAWER*, Department of Biology, Washington Square College, New York University. (Introduced by Albert S. Gordon)

Examination has been made of the changes induced, by a variety of endocrine conditions, in the numbers and morphology of peritoneal fluid mast cells of the rat. We have reported previously that adrenalectomy results in a decrease in mast cell diameter and that hypophysectomy is attended by an increase in size and by alterations in the shape of the mast cell. Recent studies, utilizing sham-operated rats and hormonal replacement treatments, have indicated that the changes following adrenalectomy are due to the surgical procedure rather than to the lack of the adrenal. The effects observed after hypophysectomy, however, cannot be attributed to the surgery *per se* and may be reversed by implantation of fresh anterior pituitary or by injections of somatotrophin (Raben-Westermeyer preparation). The experimental treatments do not affect the mast cells in mesenteric tissue spreads where these elements are found to be irregularly-shaped under all the conditions tested. The mast cells of the peritoneal and pleural fluids, however, are normally spherical or slightly ovoid and are responsive, in these sites, to a variety of experimental conditions.

[1] Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

270. *Does vitamin D act at the site of calcification?* George H. PAFF, John S. McANALLY* and William L. McLAUGHLIN*, Departments of Anatomy and Biochemistry, University of Miami School of Medicine, and the Department of Biochemistry, Hahnemann Medical College.

Blood plasma and muscle tissue extract were obtained from rachitic chicks and used as the basic media in which 8 to 9 day old embryonic chick fibulae were grown in tissue culture. Over 300 pairs of fibulae were dissected and one member of each pair was put in rachitic media which had been irradiated with ultraviolet light (varying lengths of time) or to which vitamin D_3 had been added; the other member of each pair was put in untreated rachitic media as a control. An index of the amount of calcification in each member of a pair of fibulae was obtained by comparing the alizarin receptive zone after the bones had been cultured for 4 to 8 days. Results were uniformly negative, i.e., neither irradiation nor addition of vitamin D_3 enhanced the calcification process. Only one difference was noted. In 2 of 5 groups of bones grown in media irradiated for an extended period of time, calcification was less than in those grown in non-irradiated media (possibly due to the production of toxic sterols). Needless to say we had hoped for positive results.

271. *Transformation of the golden statoblast.* James W. PAPEZ, Columbus (Ohio) State Hospital.

The golden statoblast is one of three varieties found in nervous tissue, cerebral hypophysis, and cerebrospinal fluid. Its origin seems to be from protoplast of common zoospore infestation. It is round, about six microns in diameter, with about fifteen sharp spines on its margin. It has a golden brown color suggestive of lipoprotein. Usually it contains several globules visible through its opaque surface. It resembles a crenated red blood corpuscle, from which it is hard to distinguish when its internal globules are not seen.

Transformation of golden statoblast was studied under oil immersion, phase microscope. Activity developed within; spiny processes were withdrawn, and surface membrane developed a dark granular band beneath it. At this stage it resembled a typical protoplast. Brown and yellow material was rapidly transformed into a dark, gray, mucoid substance with a strong outer membrane. Numerous fine filaments of short length protruded from the surface. Usually this change took one or more hours. On the filaments there developed one to four or more small globules. They lashed around outside of the gray sac, often for several days. They entered the sac and gradually became quiet, and shrank into small black particles in a resting stage. In this sequestered stage the zoospores persisted for several weeks during which many of the sacs shrank or folded up.

272. *Insertion of the proximal stump of a peripheral nerve into the brain of larval Amblystoma tigrinum.*[1] Jean PIATT and Donald SCOTT, Jr., Department of Anatomy, University of Pennsylvania.

The superficial ophthalmic nerve of larval Amblystoma tigrinum (ca. 50 mm length) was cut distally; the free end of the proximal portion was led through

a small hole in the cranium and inserted dorsally into the posterior third of the cerebral hemisphere. Twelve larvae were sectioned at various post-operative dates. Prior to fixation three of the oldest animals were tested physiologically for possible synaptic connections of the regenerated peripheral fibers within the brain.

Electrophysiologic exploration of both hemispheres for evidence of activity following stimulation of the implanted nerve failed to show any potentials beyond the expected passive spread of the afferent volley characteristic of a volume conductor.

The great majority of the nerve fibers failed to penetrate the brain or ran for only a few micra, ending abruptly without evidence of further growth. Only one or two fibers in each case could be traced for any distance within the brain. In one case a single fiber was traced as far as the olfactory bulb. In all animals small fascicles from the main nerve had regenerated for long distances along the meninges outside the brain, demonstrating a strong inherent capacity for regenerative growth. It is concluded that regenerating peripheral nerves of urodeles grow very poorly within the brain substance, despite the apparent lack of a glial barrier.

[1] Supported by grant B-210 from the U. S. Public Health Service.

273. Studies suggesting a possible inhibition of gonadotropic hormone effects by lymphoid tissue. Kenneth M. RICHTER, Department of Anatomy, University of Oklahoma School of Medicine.

Previous studies (Gordon, '37; Richter, '44; Turley and Richter, '41) have suggested that lymphoid tissue somehow has the capacity to exert an anti-hormonal effect. Experiments have been performed on sexually mature male guinea pigs to test empirically whether an experimental lymphoid hyperplasia (Turley and Richter, '41) could inhibit the action of toxic doses of gonadotropic hormone (antuitrin-S). (I) Ten animals received daily injections of 10 I.R.U. of antuitrin-S per 45 grams of body weight. (II) Ten animals received same antuitrin-S treatment as in group I plus 1.5 grams of aleuronat daily in their food. (III) Six untreated animals served as controls. Blood counts and body weights were recorded daily on all animals for ten days when they were sacrificed. The testes alone and the testes-plus-epididymi of each animal were weighed. The testes alone averaged .419, .29 and .329 grams-percent-body-weight in groups I, II and III respectively. The testes-plus-epididymi averaged 1.06, .7 and .73 grams-percent body weight in groups I, II and III respectively. The average daily body weight loss was 6.8 and 1.5 grams respectively in groups I and II. A characteristic leucocytosis occurred in groups I and II. The differences in genital weights between groups I and II are statistically significant. Parallel experiments employing toxic doses of Antuitrin-Growth (20 I.R.U. every other day) indicated no antagonistic action.

274. *The response of the developing forelimb of Amblystoma to implants of methylcholanthrene.* Alvin F. RIECK*,[1] Department of Physiology, Marquette University. (Introduced by Walter Zeit)

Developing right forelimb buds of *Amblystoma maculatum* and *A. opacum* (Harrison's stages 38–40) were implanted with methylcholanthrene crystals of various sizes, some implants consisted of a few large crystals, whereas, others consisted of many very small crystals with a comparable total volume. The slight solubility and length of persistence as an implant made it feasible to utilize small glass crystal implants of comparable size and volume in control experiments.

The glass implanted controls developed normal limbs in all cases.

The development of specific portions of the methylcholanthrene implanted limb buds was inhibited. The suppression was greater when an implant of many small crystals was used. The region of the limb which failed to develop was dependent upon the position of the implant. A deep implant would suppress shoulder girdle and proximal humeral regions, whereas, a more laterally placed implant would suppress distal humeral and proximal radio-ulnar structures.

From these results it is felt that the action of methylcholanthrene is not merely a mechanical block, but a direct action which is kept localized due to the limited solubility of the substance in the tissue fluids. The fact that the amount of suppression is dependent on the amount of surface areas suggests that methylcholanthrene is slightly soluble in these tissue fluids.

[1] Scholar in Cancer Research of the American Cancer Society, Inc.

275. *Autoradiographs of hamster spermatozoa with labelled phosphate (P^{32}).*[1] Paul L. RISLEY, Department of Biology, University of Oregon.

Epididymal and testicular spermatozoa of hamsters were used for autoradiographic studies following single intraperitoneal injections of P^{32} labelled phosphoric acid (radioactivity of 1 μC per gram body weight). Sperm suspensions in distilled water, and other saline solutions were tested by direct embedding on Eastman Medium Lantern Slides. After rapid drying and dark room exposure for long periods (sixty days), preparations were developed and studied with ordinary and phase microscopes.

Spermatozoa from the middle cauda epididymis gave good results in about 30% of the cases after seven days interval between injection and autopsy. About 20% gave weak reactions, and the remainder did not autograph. The intensity of the autograph in the large mid-piece regions of hamster spermatozoa indicates coincident localization of phosphate concentrations in the metabolically active and motile fraction of the sperm population. Nuclei and flagellae do not autograph. Distilled water and washed suspensions were not successful. Testicular spermatozoa give only weak reactions, and have limited motility (vibratile). Motility counts of spermatozoa from the cauda epididymis show good correlation with the autoradiographic results, indicating that the most actively motile spermatozoa are characterized by higher phosphate concentrations in the mid-piece

regions. The result is interpreted as due to surface activities of phosphate-containing epididymal secretions, rather than to incorporation of radioactive phosphate in the components of the mid-piece itself.

[1] Aided by a grant from the United States Atomic Energy Commission under Contract AT(45-1)-226.

276. *The effect of D-glucosamine HCl and related compounds on the solid form of mouse sarcoma 37 grown in vitro.* Alan RUBIN*, G. F. SPRINGER* and M. J. HOGUE, Departments of Obstetrics and Gynecology, Pediatrics and Anatomy, University of Pennsylvania.

Quastel and Cantero reported that D-glucosamine HCl inhibited growth of sarcoma 37 in mice, with vascular damage and necrosis of the tumor. The present study evaluates the effects of D-glucosamine and related compounds upon the solid form of Sa37 grown in tissue culture where vascular reaction cannot take place.

Six to 10 day old tumors of Sa37 (solid form) grown in adult, male albino C.W.F. mice were used. Hanging drop tissue cultures were made using a nutrient fluid of Gey's solution, chick embryo extract and human placental serum. The drop was coagulated with chicken plasma.

After 24 hours incubation, those cultures with good growths were selected for experimentation. To these were added nutrient fluid containing one of the following: D-glucosamine HCl, 1% and 0.5%; N-acetyl-glucosamine, 1%; glucose and ammonium chloride in equimolecular mixture, 1%; and glucose, 1% and 0.5%. As controls, cultures were grown in nutrient fluid alone. Cultures were studied 24 and 48 hours after the test solutions were added.

In 1% D-glucosamine HCl the majority of cells died. The effect of 0.5% D-glucosamine HCl was less pronounced and was comparable to 1% glucose. A 1% equimolecular mixture of glucose and ammonium chloride differed only slightly from 1% D-glucosamine HCl in its deleterious effects. One half per cent glucose and 1% N-acetyl-glucosamine had no discernible harmful effect. Controls in nutrient medium alone grew well.

277. *Lathyrism in the rat: a gross study of skeletal lesions.*[1] Elbert B. RUTH, Department of Anatomy, The Johns Hopkins University.

Weanlings, immature, and adult rats were maintained on a diet of Lathyrus odoratus seeds and Purina Lab Chow for periods less than one week to over six months. Characteristic skeletal lesions affect principal sites of muscle attachment on axial (including skull) and appendicular skeleton. Lesions first appear as surface areas of enlarged vascular foramina; progressively form irregular excavations of the osseous surface, followed by deposition of osseous elevations about excavations, finally forming exuberant osseous excrescences irregularly circumscribing lesions. Neoplastic soft tissue forms spherical or elongated tumor covering osseous lesion. Tumors about shoulder, elbow, hip, and knee joints cause characteristic modifications of posture and gait. Knee action is further modified by 90° posterior curvature of proximal end of tibia in later stages. Marked

lordosis (thoracic) and sternal deformities are common. Death after 6 months appears due to inanition as tumors about shoulder and elbow joints inhibit action of forelegs in feeding. Histological studies will appear in subsequent report.

[1] Supported by grant from National Institutes of Health, Public Health Service.

278. *The accessory body in nerve cell nuclei of the cat.*[1] Bertha K. SCHURMAN*, Department of Microscopic Anatomy, University of Western Ontario. (Introduced by Murray L. Barr)

Twenty-nine regions of the nervous system were studied in three female and three male cats. Sections were stained by Nauta's silver nitrate method for the accessory body, and with cresyl echt violet for the sex chromatin.

The accessory body of Cajal is a spherical, argyrophil body, less than in 1 μ in diameter, and usually free in the nucleoplasm. It is seen in about three-fourths of nuclei in most types of neurons. There is no difference between males and females with respect to the incidence or size of the accessory body. It is not often seen in dorsal root ganglion cells, and is encountered in less than 5% of nuclei in sympathetic chain ganglia and the dorsal vagal nucleus. The accessory body varies in size from one type of neuron to another, being large in cord cells and small in cells of the caudate nucleus. The accessory body differs from the sex chromatin in that it is identical in males and females, in its staining properties, position and, to a lesser degree, size and shape. Nothing seems to be known concerning the relation of the accessory body to other nuclear structures or its role in the physiology of the cell.

[1] Supported by National Health Grants of Canada.

279. *Giant nucleolar-like structures in the anterior lobe of the hypophysis of the gonadectomized hamster.*[1] Barbara Jo SERBER*, Department of Anatomy, Columbia University. (Introduced by Frederic J. Agate)

Giant nucleolar-like structures have been reported in the human hypophysis by Cavallero (Arch. Path., 44: 639–645, 1947). In the course of a cytological study on the anterior lobe of the hypophysis of the gonadectomized hamster, similar structures have come to our attention.

No giant nucleolar-like structures were seen in the pituitary glands of 17 control hamsters. Glands of three animals, sacrificed during the first 4 months following gonadectomy, averaged only one of these structures per two or three sagittal sections (3 μ), while hypophyses of 5 hamsters, gonadectomized from 4 to 6 months, averaged from 6.5 to 24.5 per section. They were less frequent in glands of 4 animals 6 to 12 months post-operatively, averaging below 5 per section, and extremely rare (less than one per section) in glands of 5 hamsters 12 to 18 months following gonadectomy.

These nucleoli, observed only in basophils, are round to oval, appear slightly granular, and have an outer dark limiting border. They are centrally or slightly

eccentrically placed in the nucleus. Their size is variable; some are relatively small, while others almost fill the nucleus.

The structures color weakly with Schiff reagent following periodic acid, stain faintly with aldehyde-fuchsin, also with the aniline blue of Masson, exhibit no metachromasia with toluidin blue, and give a negative Feulgen reaction.

[1] Supported by U. S. Public Health Service grant RG-1447(C2).

280. Nitrogen metabolism in embryonic tissues of the chick. Mary Elizabeth SHONTZ* and Janice MASON*, Winship Clinic, Emory University School of Medicine. (Introduced by J. Szepsenwol)

Changes in total nitrogen, non-protein nitrogen and its fractions were studied in liver, brain, chorio-allantoic membrane, heart, digestive tract and skeletal muscle of chick embryos between the 8th and 21st days of incubation. For total nitrogen the tissue was ashed with sulfuric acid and nitrogen determined by the micro-Kjeldahl distillation and titration method. The acetic acid deproteinization method of Russell was used for NPN. The filtrate was divided into aliquots for determination of total NPN, peptide, amino acid, ammonia and amide nitrogen. The methods used were: the micro-Kjeldahl distillation and titration method for NPN, the method of Frame, Vilhelmi and Russell for amino acid and peptide (JBC, 156, 1943), the Conway method for ammonia, and the method of Vickery, Pucher and Clark for amide nitrogen (Biochem. J., 29, 1935).

Total nitrogen shows little changes in brain and liver. It increases considerably in heart, digestive tract and particularly in skeletal muscle, where it more than doubles its concentration between the 11th and 21st days of incubation. In the chorio-allantoic membrane total nitrogen also increases to some extent. NPN follows a parallel course with total nitrogen, increasing simultaneously. The changes are due mainly to peptides and amino acids, the major constituents of the NPN. The amide and ammonia nitrogen is relatively low in all the tissues and it does not show any major changes throughout development.

281. Petechial susceptibility of the hamster cheek pouch subjected to negative pressure.[1] Maurice H. SHULMAN*, Elmer B. MODE*, Roma KAGAN* and George P. FULTON, Departments of Biology and Mathematics, Boston University.

Spontaneous petechiae occur in the cheek pouch of the hamster, *Mesocricetus auratus*, following exposure to ionizing radiation, treatment with traumatic chemicals (moccasin venom, turpentine) and during infection (Lutz and co-workers, 1953, Fed. Proc., 12: 92). The susceptibility to petechial formation is tested under nembutal anesthesia by negative pressure from a suction cup applied to the cheek pouch. A standard value for normal untreated hamsters was obtained by testing both cheek pouches of 30 males and 30 females. Negative pressure (200 mm. Hg) was applied for one minute and the number of petechiae counted. The data was analyzed statistically. The distribution of the number of petechiae was somewhat suggestive of the Poisson distribution. A mean value of 1.7 petechiae (standard deviation 2.0) was obtained. A slightly higher value, 2.6

petechiae (standard deviation 2.9) was found for 20 hamsters (10 of each sex) tested every five days for 30 days. No significant differences occurred in mean petechial counts between sexes or right and left pouches. Confidence limits for the mean values are in use as standards for detection of vascular weakness following irradiation and for evaluation of substances reported to be protective.

[1] Supported by a grant from the Atomic Energy Commission.

282. Electron microscopic studies of the glomerulus in nephritic mice of the NH strain. Parke H. SIMER, Department of Anatomy, University of Illinois College of Medicine. .

Electron photomicrographs of renal glomeruli of nephritic mice have been prepared. Glomerulonephritis with albuminuria and edema occur spontaneously in one-fourth to one-third of the females of this strain and is induced precociously in females as well as in males by multiple injections of urethane, horse serum or nephrotoxic serum prepared in rabbits.

The glomerular capillaries of chronically nephritic mice exhibiting albuminuria show marked modification from their normal structure. The endothelial cytoplasm thickens irregularly and may increase so that it appears to reduce the size of the capillary lumen. The basement membrane increases from a normal thickness of .15 micron to as much as .30 micron. The pedicels in the normal are evenly spaced, extending from the basement membrane into the space between it and the cytoplasm of adjacent epithelial cells. They average about .30 micron in length but may be much longer and appear to be continuous with epithelial cell cytoplasm. In capillaries of nephritic glomeruli, the pedicels are wider, more unevenly spaced, and less evident. The cytoplasm of epithelial cells establishes a more intimate relationship with the basement membrane. The nephritic glomerulus is compact and lobulated and is more cellular and less vascular than in the normal. The capillaries are infrequent with small lumina and walls increased to three times their normal thickness.

283. Quantitative studies on acid phosphatase in different regions of the central nervous system.[1] Wilbur K. SMITH, Irene MIALE* and Alexandra FELDMAHN*, Department of Anatomy, The University of Rochester School of Medicine and Dentistry.

Tissue samples from various parts of the central nervous system of adult cats and kittens were analyzed for acid phosphatase, using the method of Bessey, Lowry and Brock (1946). In adult cats, the cerebellar cortex yielded the highest value. Cerebral cortex and caudate nucleus were lower, the amount in these two regions corresponding closely. Cerebral white matter was found to contain about one half as much enzyme as the cerebral cortex. White matter from the spinal cord and peripheral nerve yielded the lowest values, approximately one half of the amount found in the cerebral white matter. The amount of acid phosphatase appeared to be correlated with the number of nerve cell bodies present. A striking feature of the analyses was the remarkable uniformity of the values as determined for the same region in different animals. For example, values for the

cerebral cortex in five animals ranged from 0.61 to 0.64 millimol units per milligram of fresh tissue.

Analyses of tissue from four day old kittens showed acid phosphatase content in the cerebellum and cerebral cortex to be considerably less than in mature animals. From the third or fourth week post-natally, the enzyme values approximated those found in the adult.

[1] Supported from a grant from the USPHS.

284. Radioactive phosphate (P^{32}) in the hamster testis and epididymis.[1] A. L. SODERWALL* and Alan L. CHAIMOV*, Department of Biology, University of Oregon. (Introduced by Paul L. Risley)

Adult male hamsters received single intraperitoneal injections of radioactive phosphate (P^{32}; 1 μC/gram body weight). Forty-eight hours later, testes and epididymides were assayed for radioactivity. Testis uptake and turnover is fairly stable for three to six days. Increasing variability with time, and increasing P^{32} concentrations are observed to the thirteenth day. Microscopic study indicates testis radiation damage to be correlated with increasing P^{32} concentrations.

Seven fractions of each epididymis were tested as follows: upper and lower caput, upper and lower body, and upper, middle, and lower cauda. Lowest values occur in the testis and lower cauda, coincident with high sperm concentrations. Highest values appear in the lower body and upper cauda, where epididymal secretions are accumulated, and alterations in sperm behavior and motility appear. The middle cauda is characterized by decreasing concentrations. The upper caput including vasa efferentia shows increased P^{32} content over the testis, but has less than the lower caput and upper body. Between 24 and 48 hours, a shift of the highest concentration noted from the lower caput to the upper cauda.

Epididymides of unilateral castrates (without spermatozoa) gave uniform high phosphate levels in the entire cauda of the castrate side. Low phosphate values occur in the middle and lower cauda of the normal side, indicating greater phosphate turnover where spermatozoa are concentrated.

[1] Aided by a grant from the United States Atomic Energy Commission under Contract AT(45–1)–226.

285. Histories of experimentally-induced nerve plexuses involving sensory vagus and spinal fibers.[1] Carl Caskey SPEIDEL, Department of Anatomy, University of Virginia.

The dorsal rami of the vagus nerves extending longitudinally in the dorsal tail fin of the frog tadpole lie superficially to the spinal nerves which they traverse. The two are usually anatomically separate as well as functionally different. The vagus fibers carry afferent impulses from the special sense organs of the lateral line; the spinal fibers carry general somatic afferent impulses from the skin. Suitable nerve transections or fin tissue extirpations induced some plexuses between vagus and spinal nerves, thus decreasing their original anatomical separateness. This was noted between (1) peripheral degenerating stumps, (2) central irritated stumps, (3) peripheral vagus stumps and uncut spinal nerves, (4) peripheral spinal stumps and uncut vagus nerves, (5) regenerating vagus and

regenerating spinal fibers in wound zones. Sheath cells appeared to be the chief agents in establishing such connections. Further nerve transections were made successively at strategic sites in many cases and the adjustments during recovery were recorded. These observations indicated that vagus and spinal fibers though closely intermingled in single nerve trunks remained specific as to their end distribution. Spinal nerve fibers innervated the skin only, and never lateral line sense organs; vagus fibers innervated lateral line organs only.

[1] This investigation was supported by a research grant (PHS B-359) from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service.

286. Observations on the relation of an afferent volley to the strychnine discharge in the cerebral cortex of the cat.[1] Jerome SUTIN*, Department of Anatomy, University of Minnesota. (Introduced by Berry Campbell)

Microelectrode analysis of the strychninized cerebral cortex indicates that the infragranular portion of this structure is active 20 to 40 milliseconds before the supragranular layer is involved. These two components of the strychnine response may be selectively abolished by topical or subcortical placement of formalin or narcotics.

When potentials are evoked from a strychninized portion of the cortex by contralateral stimulation of a symmetrical cortical point, the latency of the afferent volley arriving at the drugged region is consistently within the range of 4–6 milliseconds, while the beginning of the strychnine response has a latency which varies from 5 to 80 milliseconds, depending upon the stimulus intensity used.

Recordings made with a condenser coupled amplifier with a time constant of 0.5 seconds show no electrical activity during this latent period between the arrival of the afferent volley and the strychnine discharge.

If a high voltage discharge is induced in a region of the cortex by supramaximal stimulation of the opposite cortical region and strychnine subsequently placed on the discharging cortex, the latency of the afferent volley remains unchanged while that of the response increases by 10 milliseconds.

[1] Aided by a grant from the national Institute of Neurological Disease and Blindness, of the National Institutes of Health.

287. The effect of contractures on electromyographic activity in spastic hemiplegia. C. A. SWINYARD, Department of Anatomy, University of Utah College of Medicine.

[No abstract.]

288. Tissue culture tests of viability of frozen and pretreated frozen skin grafts.[1] A. Cecil TAYLOR, Robert GERSTNER* and Gloria ROLLE*, Department of Anatomy, College of Dentistry, New York University.

Viability and "take" of untreated frozen and ethylene glycol (EG) pretreated frozen skin autografts in mice were determined by tissue culture methods. The use of arbitrary gradings (1 to 4) based on cultures of unfrozen, untreated control skin autografts enabled direct comparison to be made between experimental grafts

and controls. Biopsies from autografts were taken for culturing 1, 2, 3, 4, 8 and 10 days after operating. 436 fragments were cultured.

Biopsies taken one day after grafting, from both control and EG pretreated frozen grafts, when cultured, showed cellular outgrowth (somewhat greater in controls). No cells, however, were seen in the cultures of biopsies from untreated frozen grafts. Three days after grafting, cultures taken from control and EG pretreated frozen autografts were equal in extent of cellular outgrowth and were graded 3; cultures from frozen untreated grafts showed outgrowth of less than grade 1. Eight days after grafting, cultures from all autografts were graded 3. Preliminary tests with similarly treated tissues encased in cellophane sacs (dializing membrane) and implanted in the body cavities of mice for 1 day duplicated the results described above as seen one day after grafting.

Suggested interpretations: Freezing without pretreatment (1) kills the cells of the graft; the activity seen on the 8th day being due to cells which have invaded from the host, or (2) inhibits and delays the activity of surviving cells.

[1] Supported by a grant from the American Cancer Society on recommendation of the National Research Council, Committee on Growth to the Department of Anatomy, Dental College, and by a grant from the Atomic Energy Commission to the Plastic Surgery Unit, Department of Surgery, College of Medicine, New York University.

289. *Pulmonary lymphatics.*[1] Charles E. TOBIN, Department of Anatomy, The University of Rochester School of Medicine and Dentistry.

Studies were made of lymphatic vessels, valves and nodes from microscopic and macroscopic sections of normal fetal, neo-natal and adult lungs; injections of various media into the pleural lymphatics of normal fetal, neo-natal and pathologic adult lungs; and injections of India Ink or Direct Sky Blue into the bronchi or pleura of living cats and rats.

These observations indicate that the normal lymphatic drainage from the larger respiratory passages is via lymphatics along the bronchi and pulmonary artery towards the hilus, whereas that from the smaller airways and visceral pleura is also towards the hilus but along channels around the pulmonary veins.

If the hilar and more distal nodes become occluded by growth of fibrous tissue or accumulations of foreign material, or if larger lymphatic channels are occluded by pressure from adjacent growths, pleural lymphatics, by their connections with channels from within the lung, may convey lymph or foreign material around lobules, segments or lobes to the hilar region. The pleural lymphatics of adult lungs could be injected only in such cases.

Valves which are normally present in the hilar and pleural regions are numerous in fetal and neo-natal lungs but their number decreases with increasing age.

[1] This investigation was supported by a research grant (C-1946) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

290. *Alkaline phosphatase content of the rat pituitary.* Edgar A. TONNA*, Department of Biology, Washington Square College, New York University. (Introduced by Albert S. Gordon)

Study was made of the localization and distribution of alkaline phosphatase (Glick, 1949) in the rat pituitary. The animals were separated into 4 groups: normal males, normal females in estrus, non-estrous females and ovariectomized

animals (used 2 months post-operatively). No significant variations were noted in the alkaline phosphatase activity of the different cell types within the pituitary of normal male and female rats. The pars anterior of estrous females revealed a slight decrease in alkaline phosphatase activity as compared to the non-estrous group. Castration resulted in an increase in alkaline phosphatase activity particularly in the basophiles, cells known to be associated with the production of excess FSH at this time. The enzymatic activity of the pars intermedia was slightly greater in female than in male rats. The pars intermedia did not reveal any change in alkaline phosphatase content during the various stages of the female reproductive cycle. A slight increase in activity, however, was noted in the pars intermedia of spayed females. The alkaline phosphatase activity of the pars nervosa was similar for males, non-estrous females and ovariectomized animals, although there was a slight reduction in activity in the estrous group. It is concluded that the alkaline phosphatase reaction may serve as a useful index of the relative metabolic state of the cellular types of the hypophysis.

291. A concept of steroid hormone-vitamin A-carcinogen interrelations. B. Lionel TRUSCOTT, Department of Anatomy, School of Medicine, University of North Carolina.

An antagonistic interrelation between estrogen and vitamin A has been repeatedly demonstrated; similar data have been presented which would link certain carcinogens with this vitamin. In the present study of the hepatic stores of vitamin A, about 500 mice of the C_{57} strain were utilized; these were divided into intact and castrate series, and each series further separated into untreated and methylcholanthrene-injected groups. Castration resulted in hepatic reserves of the vitamin which were almost three times those of the intact, untreated animal; this difference was further enhanced by subcutaneous injection of methylcholanthrene. Although methylcholanthrene tends to depress hepatic stores of vitamin A in the intact animal, these changes are not striking. Neither body weight, liver weight nor food intake (measured in groups on calorie-restricted diets) reflected the marked changes noted in hepatic levels of the vitamin. On the basis of these and other studies by the author and other studies by the author and other workers, it is suggested that vitamin A is part of a metabolic cycle which includes cholesterol, squalene, steroid hormones and carcinogens; a marked change in any of these components will be reflected by changes, often compensatory, in the relative proportion of one or more of the other components in the cycle. This concept, elaborated from an earlier proposal (Truscott, '43, '50) will receive detailed consideration in a subsequent paper.

292. Hyperthermal and surgical lesions of the cerebral cortex of the cat. B. Lionel TRUSCOTT, Department of Anatomy, School of Medicine, University of North Carolina.

Studies of cortical association pathways by degeneration techniques are hindered by concomitant injury to adjacent, extraneous fibers; degeneration of the latter obscure visualization of the pathways under consideration. The author has attempted to obviate these difficulties by lesions of selective depth and minimal

inflammatory reaction. These preliminary experiments were performed on cats in which cortical lesions were produced by cautery, surgical ablation and hyperthermy; the latter was accomplished by a specially-shaped copper tube of known basal dimensions, and heated to 80°C. by a continual flow of water through the tube. Injuries by ablation and cautery could not be controlled in depth and were accompanied by a moderate inflammatory reaction. Hyperthermal lesions could be partially controlled in depth, and were best visualized when the exposure time was greater than ten seconds and at least two hours had elapsed before fixation of the tissue. Inflammatory reactions and extravasation of blood are common in hyperthermal lesions which are thus far from ideal. It is hoped that hypotherml injuries, now being performed, will provide a more suitable method of producing degeneration of association pathways.

293. The relation between temperature and conduction velocity in nerve fibers of different sizes. R. S. TURNER, Department of Anatomy, Stanford University.

Individual responses of single nerve fibers of significantly different size have been studied at different temperatures in the myelinated medial and lateral giant fibers of *Lumbricus*. From 100 measurements made on such preparations the following results were obtained: Mean conduction velocity of the medial giant fibers at 22.3°C. was 18.84 meters per second; mean conduction velocity of the laterals at 22.3°C. was 9.62 m.p.s. At a mean temperature of 8.2°C. the conduction velocities were respectively 10.77 m.p.s. for the medial and 5.69 m.p.s. for the lateral giant fibers. The conduction velocity was thus reduced proportionately in the two groups, that of the medial fibers by 42.8%, that of the lateral fibers by 40.9%. The difference between these two figures is not statistically significant. In the temperature range of 5.0°–25.0°C. the mean Q_{10} for conduction velocity of the medial fibers is 1.49, that for the lateral fibers is 1.45. The mean diameter of the medial giant fibers measured in this laboratory was 62.7 micra; the mean diameter of the laterals was 34.6 micra. Conduction velocity appears to vary directly with fiber diameter. With regard to current theories of conduction these nerves probably occupy a position intermediate between unmyelinated fibers and myelinated fibers with nodes of Ranvier.

294. Slow high potential waves produced by electrolysis. A. VAZ FERREIRA and C. GALEANO*, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay.

Small electrolytic lesions were produced in the thalamus and some other structures of the brain of young (average weight: 200 gm) albino rats under Nembutal anaesthesia by means of Krieg's stereotaxic machine, with the positive pole, using an electrical current of two milliamperes for 5 seconds through an insulated copper electrode of a 220 μ diameter. The electrical nervous activity was recorded from the copper electrode itself before and after the electrolysis by means of an eight-channel Grass Electroencephalograph, model 111 D: the outstanding feature after electrolysis was the appearance, in a high percentage of cases, of slow (frequently 2½ c/s) high voltage waves, independent of cardiac and respira-

tory movements, which after about roughly one hour were gradually replaced again by a faster and lower voltage activity. If the rat is dead, or very depressed by an overdose of Nembutal or by bleeding, these slow waves do not appear. No similar activity is obtained by electrolysis of the muscle.

When the electrolysis is done with the negative pole, the slow waves activity is more irregular and lasts a shorter time.

By using multiple electrodes it is possible to localize the point of origin of the slow waves by the phase reversal. A comparison with other electrodes (silver, nichrome, etc.) is in progress; also the study of the anatomy of the lesions.

295. A study of the human medial peroneal cutaneous nerve, a new name proposed for the peroneal anastomotic nerve. D. D. WILLIAMS*, Departments of Physiology and Zoology, The University of Illinois. (Introduced by F. B. Adamstone)

A study of the medial peroneal cutaneous nerve was made over a two year period to determine its origin. Of 154 cadavers (128 caucasians; 25 negroes; 1 mongolian) 141 were males and 13 females. The observations include those from 257 lower extremities, the nerve in 51 cases being mutilated before its status could be definitely determined. The medial peroneal cutaneous nerve arose from the common peroneal nerve in 239 cases (93%), and from the lateral sural cutaneous in 3 cases (1.17%). In 4 cases (1.55%) the medial peroneal cutaneous nerve and the lateral sural cutaneous nerve arose from a common trunk from the common peroneal nerve. No true sural nerve occurred in 42 cases (16.34%), and in 41 of these cases (15.95%) the medial sural cutaneous nerve acted in the capacity of the sural nerve while in only one case (0.39%) the medial peroneal cutaneous nerve served as the sural nerve. In 11 cases (4.28%) the medial peroneal cutaneous nerve was absent, and the medial sural cutaneous nerve was absent in only one case (0.39%).

The new term, medial peroneal cutaneous, is being proposed because the terms "anastomotic" and "communicating" do not apply.

296. Effects of cortisone on hepatic parenchyma, myocardium, and body weight of mice. W. Lane WILLIAMS, Department of Anatomy, University of Minnesota.

For 6 days, young adult mice received cortisone daily at a dose level of 12.5 mg/100 gm of initial body weight, by either intramuscular or intraperitoneal injection. These mice were killed 24 hours after the last (sixth) injection of the hormone. Intramuscular cortisone produced a 22% loss in body weight. After intraperitoneal injection of cortisone for the same period, body weight was 15% less than the initial level. Hepatic parenchymal cells of both groups of mice showed loss of cytoplasmic ribonucleic acid (basophilic staining-ribonuclease hydrolysis technic), with the greater depletion occurring in the intramuscular group. The amount of myocardial and hepatic parenchymal glycogen (PA-S technic) was essentially the same in the intramuscular and intraperitoneal groups, and was not increased as much as in rats and rabbits receiving equivalent amounts of cortisone per 100 gm of body weight.

Other mice similarly received cortisone. Members of this group were killed at intervals of 7, 10, 20, and 23 days after the sixth injection. The intraperitoneal group attained pre-injection body weights within 20 days. In contrast, 23 days after termination of injection of cortisone the body weights of the intramuscular group remained 4.3% less than the initial level.

297. *Selective staining of the otolithic membranes, cupulae and tectorial membrane of the inner ear.*[1] George B. WISLOCKI and Aaron J. LADMAN*,[2] Department of Anatomy, Harvard Medical School.

The tectorial membrane, gelatinous substance of the otolithic membranes and cupulae of the internal ear (mouse, rat, human fetus) are intensely and selectively stained by Gomori's chrome alum-hematoxylin and aldehyde-fuchsin stains, and react strongly with the periodic acid-Schiff reagent following exposure to saliva. The staining of these structures is similar to that of the ciliary zonula, secretory granules of the subcommissural organ, Reissner's fiber and hypophysial Herring substance (cf. Wislocki and Leduc, 1952, Jour. Comp. Neurol.).

The positive periodic acid-Schiff reaction of these neural structures indicates the presence of a mucopolysaccharide, but the absence of metachromasia with toluidin blue and the relatively faint basophilic staining with methylene blue below pH 5 do not suggest a sulfated mucopolysaccharide. A moderate reaction for protein-bound sulfhydryl and disulfide groups is revealed by Barrnett and Seligman's methods. The structures react faintly with Weigert's resorcin fuchsin elastic tissue stain, excepting the tectorial membrane which stains moderately intensely brown. The sum of these reactions indicates the presence of a protein with distinctive properties.

The cytoplasm and hairs of the developing sensory cells of Corti's organ stain quite intensely by the disulfide procedure, a result indicating a protein rich in disulfide groups. On the other hand, the hairs and flagella of the otolithic membranes and cupulae stain faintly.

[1] Aided by a grant from the Eugene Higgins Trust.
[2] Fellow of the American Cancer Society.

298. *Sexual dimorphism in the Harderian gland of the hamster (Cricetus auratus).*[1] George W. WOOLLEY and Joanne WORLEY*, Division of Steroid Biology, Sloan-Kettering Institute.

A sexual dimorphism related to the presence or absence of black-brown pigment in the Harderian gland of the golden hamster has been described. In the present series this sexual dimorphism was evident. We also observed that gonadectomized males and females 18–24 months of age had granules similar to those observed in intact females. In 14-day experiments, granules were present in males treated with estrogens and minute or absent in females treated with testosterone propionate. The granules were dark colored following fixation in Zenker-formol and light brown following fixation in Vandergrift's fluid.

A second sex-influenced difference, heretofore unreported, concerns the structure of the cells of the tubules. Typically, in the female, the cytoplasm contains minute droplets which appear in section as small vacuoles, separated from each

other by acidophilic cytoplasm. In the male, a large number of these cells contain uniformly large rather than small vacuoles. These large-vacuole cells are either absent or occur infrequently in females. Females and males gonadectomized for 30 days tended to be similar to females. The large-vacuole cells were irregularly vacuolated and regressed in males treated for 10 days with estrogenic hormones and appeared in females similarly treated with androgenic hormones. This pronounced dimorphism was not found in the rabbit, rat, mouse, guinea pig, or Chinese hamster.

[1] Aided by United States Public Health Service Grant C-1796 (C), and by the American Cancer Society.

299. The development of muscles and tendons in nerveless and weakly innervated chick limb grafts.[1] Ruby A. WORTHAM* and Herbert L. EASTLICK, Departments of Zoology, University of Idaho and the State College of Washington.

In normal embryos differentiation of premuscle masses into individual muscles begins during the sixth day and is almost completed by the eighth day. The thigh muscles begin their differentiation earlier than those of the lower leg while the short toe muscles do not start to form until the seventh day. The formation of individual muscles is accomplished primarily by longitudinal splitting of the masses, after which the tendons form at their distal ends. The respective tendons continue their development and become inserted on the bone, normally between the ninth and eleventh days. Development of muscles and tendons in the grafts follows the same general pattern as that in controls but in every case the graft is retarded 24 to 48 hours. It is possible to identify many of the larger muscles together with their tendons. Development continues essentially in a normal manner, for several days and the tendons become more compact and in many instances appear to insert on the bone. On the tenth day or shortly thereafter the muscles begin to lose their individuality and become exceedingly difficult to recognize. However, many of the tendons, particularly the large flexors and extensors, can still be observed. In most instances, they compare in histological structure and position with those of the controls.

[1] Supported in part by the State College of Washington Committee on Research.

300. The tectorial membrane as a sound receptor. M. Wharton YOUNG, Chiba Medical College, Japan and Howard Medical College.

The cochlea may be considered as the special somatic afferent receptor for the acoustic nerve. Many sensory terminals are formed by peripheral nerve filaments associated with epithelial structures or completely encapsulated. The hair cells of Corti's organ are true neurons synaptically joined to the bipolar cells of the spiral ganglion while their terminal hairs extend upward into the overlying tectorial membrane and thus contribute to its fibrillar structure. This continuity is well established in the embryo where the tectorial membrane is secreted by the epithelial cells of Corti's organ and it may be retained in carefully prepared adult specimens. The separation usually seen may be an artefact resulting from fixation and shrinkage. In general the afferent path begins at the "end" of the

sensory neuron where the stimulus is converted into a nerve impulse, thus the tectorial membrane marks the peripheral end of the auditory path for the reception of sound. This membrane with its compartmental divisions, its varied width, its colloidal nature and its delicate fibrillar structure is better adapted to displacements of 10^{-10} cms. than the rigid basilar membrane which is devoid of nerve endings. The colloidal reaction to sound may be chemical, as in the retina, and vitamin A has clinically improved deafness as well as night blindness.

DEMONSTRATIONS

D 52. Functional anatomy of the stimulated lumbar sympathetic trunk in man. W. F. ALEXANDER, W. C. RANDALL*, J. W. COX* and K. B. COLDWATER*, Departments of Anatomy and Physiology, St. Louis University School of Medicine and the Surgical Service, Veterans Administration Hospital, Jefferson Barracks.

The lumbar sympathetic trunks were mapped and stimulated prior to extirpation in patients subjected to operation for various causes. Sudomotor responses in the lower extremity were recorded by the starch paper and iodine technic. Pilomotor responses were observed. The trunks were fixed in formol-saline, stained with osmic acid or Sudan Black B and sectioned serially. Each sympathetic trunk was accurately oriented on an X-ray which was taken postoperatively. The trunk was drawn on the X-ray using the silver clips placed in position on the trunk and its rami at the time of surgery for accurate localization. Fiber analysis of the sympathetic trunk and its rami was made and this was correlated with the functional response from the specific site of stimulation.

No segmental pattern could be observed from the sudomotor responses after stimulation of either the trunk or its pre- or postganglionic communications. Stimulation of the trunk at the L1 vertebral level often produced sweating responses in the entire lower extremity. Occasionally the sweat glands in the distal part of the lower extremity reacted only when the trunk was stimulated at the level of L4 or even L5. Occasionally a communicating ramus was observed to reach the trunk at the level of L4 or L5 which upon stimulation produced sweating in the lower extremity. Small myelinated fibers were observed in these rami.

D 40. Degeneration in the lateral hypothalamic area after lesions in the frontal tip of the cat's brain.[1] J. AUER, Department of Anatomy, Faculty of Medicine, University of Ottawa.

The tip of the frontal lobe has been coagulated in a series of cats. The cats were sacrificed five days after the operation and the brains were removed and treated either with the Auer-Di Virgilio silver technique or with the selective impregnation method of Nauta. The observation on boutons in the lateral hypothalamic area, reported in a preliminary experiment a year ago, could be verified with the former technique. The selective impregnation of degenerating fibers in the Nauta technique has made it possible to analyze in detail the consequences in the hypothalamus of a lesion in the frontal tip. Pertinent sections of both

techniques showing degeneration in the hypothalamus will be demonstrated. The fact that normal fibers are not entirely left unstained has proven to be a disadvantage with the selective impregnation. An unbroken fiber showing swellings is not an indication of degeneration.

[1] Supported by a grant of the National Research Council of Canada.

D 31. Vascular lesions in the testes of hamsters treated with stilbestrol and DOCA or stilbestrol and progesterone. R. L. BACON, H. KIRKMAN and M. GOTTLIEB*, Departments of Anatomy, The Johns Hopkins University, Stanford University, and the University of Tennessee.

Eight male golden hamsters received stilbestrol and desoxycorticosterone acetate, and 9 others received stilbestrol and progesterone from 20 mg pellets of the pure substances implanted subcutaneously between the scapulae. Treatment was begun at or about 50 days of age, and ranged in duration from 196 to 435 days. Sections of the testes of all of the first group and of 5 of the second group showed arteriolar changes ranging in severity from excessive intimal thickening through thrombosis to complete fibrous replacement. Some damaged areas appeared to have been recanalized. Mallory's connective tissue stain colored the thickened intima bright red. Mallory's method for the demonstration of hyalin degeneration with phloxine gave the characteristic red color of this reaction. Staining of the same sections by Verhoeff's technic for elastin demonstrated that, except for the most advanced stages of vascular breakdown, the hyalin deposition was limited to the tunica intima. The testes of untreated animals and of those treated with stilbestrol alone did not show these changes.

D 55. Development of the visual pathway in humans. A. N. BARBER* and G. N. RONSTROM, Departments of Pathology and Anatomy, Louisiana State University School of Medicine.

Selected photomicrographs of serial sections of human embryos and fetuses show the development of the optic nerves and optic chiasma from four weeks to birth. The relation of the developing visual pathway to the third ventricle is also illustrated. The nerve fibers from the ganglion cells in the retina reach the brain by the 18 mm. stage and fill the marginal layer between the two optic stalks by the 25 mm. stage at which time a true chiasma can be said to exist. Continued growth of the optic nerve fibers reduces the cavity of the recessus opticus but a portion of the recess persists at birth. Developmental abnormalities of the brain in several human embryos are presented.

D 29. Studies on the nebenkern, a mitochondrial body in the germ cells of certain animals. H. W. BEAMS, T. N. TAHMISIAN*, R. L. DEVINE* and L. E. ROTH*, Department of Zoology, State University of Iowa and

Division of Biological and Medical Research, Argonne National Laboratory.

The nebenkern in the spermatids of certain animals is said to be formed by the fusion of mitochondria. When mature it is composed of a series of concentric

lamellae of about 400 Å in width. The space between the lamellae is composed of a relatively structureless matrix. The arrangement of the lamellae is similar to that of the layers in an onion.

D 54. Control of cell number and size in the lateral motor column of Rana pipiens. Allan R. BEAUDOIN*, Department of Zoology, State University of Iowa. (Introduced by Jerry J. Kollros)

The differentiation of lateral motor cells in the spinal cord of Rana pipiens was observed under normal and experimental conditions. Limb ablations at stages II–III resulted in an ipsilateral delay in differentiation of these motor cells and a loss of 82% of the motor cells by stage XVI, 55 days after operation. Complete lack of the motor column was not observed. Following limb ablation, immersion in thyroxin solutions at stages V, and IX–XII, produced a more rapid loss of motor cells on the operated side. By the time stage XIII was reached, 43 days after operation, a reduction of 60% had occurred. Unilateral stimulation of hind limb growth was obtained by implantation of thyroxin-cholesterol pellets into the hind limb. Ipsilateral increase in motor cell size was obtained in animals with pellets located either in the hind limb or adjacent to the cord. Motor cell size was determined by nuclear measurements. There was no advance in stage of the motor cells beyond the stage level of the limb, even though in treated animals limb lengths varied greatly for the same stage. It is concluded that thyroxin exerts a direct as well as an indirect effect upon the size of motor neurons in the lateral motor column of Rana pipiens tadpoles.

D 47. Some applications for a new silver preparation useful in the silver-on-the-slide staining of paraffin embedded nervous tissue. James O. BROWN and J. P. M. VOGELAAR*, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

Preliminary results in staining through the use of a new silver preparation, sought to replace pre-war protargol when the latter was no longer available, have already been demonstrated (Brown and Vogelaar, '53). This new silver compound has been named Colosyn by the author (J.O.B.).

The present report concerns additional neurohistological observations of tissues stained with this new silver compound. The investigation has clearly shown that Colosyn measures up to the requirements for a protargol substitute, being perhaps better than protargol in some instances. Procedures for using Colosyn will be presented together with photomicrographs reproduced on Kodachrome slides illustrating some of the results.

D 7. Tumor growth after intraembryonic transplantation in the 2½-day chick.[1] Elmer D. BUEKER, Department of Anatomy, University of Missouri.

The following transmissable and transplantable mouse tumors (I, 37, 180, T241, Ma387, A274, S635, S637, Ds2, Ds3, Ds4, MCI, Ca15, C987) were implanted and grown in the limb fields of chick embryos. Growth effects of mouse sarcomas on sympathetic and spinal ganglia of the chick will be demonstrated.

[1] Aided by a grant from the Division of Research Grants and Fellowships of the National Institute of Health.

D 10. Induction by ultraviolet of regenerative activity in unamputated Urodele limbs.[1] E. G. BUTLER and H. F. BLUM, Department of Biology, Princeton University.

The limb of larval urodeles, *Amblystoma punctatum* and *A. opacum*, has been subjected to single doses of localized ultraviolet radiation, with effective wavelengths .313 μ and less. In administering the radiation the entire animal was shielded, except for small areas of the limb. Radiation of the carpal region results in the formation of supernumerary digits. When the elbow alone is radiated, a supernumerary hand develops at this level, complete with new carpal, metacarpal and phalangeal skeletal elements. Radiation of the shoulder results in the superregeneration of limb structures from this area. Regenerative activity following radiation becomes evident only at the end of a period of from five to six weeks after the administration of the single dose. Supernumerary growths appear in a high percentage of cases when the animals are kept in the dark after radiation. When radiated animals are kept in light of wave-lengths $\sim$ .03 to .07 μ subsequent to radiation, photorecovery takes place and few supernumerary growths occur.

[1] Supported by a research grant (C-1499) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

D 49. The effects of intense sound on the cochlea.[1] W. P. COVELL, C. A. SMITH and D. H. ELDREDGE*, Department of Otolaryngology, Washington University School of Medicine and Central Institute for the Deaf.

Serial sections of 288 guinea pig ears exposed to frequencies ranging from 1,000 to 40,000 cps at high intensities for a few seconds to 4 minutes were studied to determine the extent and degree of injury produced in the cochlea. The animals were allowed to survive for varying periods up to 4 months. Sound source for most of the experiments was a specially constructed siren capable of producing sound pressures in excess of 150 db in the range of 400 to 30,000 cps. For exposures between 125 and 150 db (ref. 0.0002 microbars) marked or severe injury to the organ of Corti was in fair agreement with localization of frequencies along the basilar membrane. For sound pressures above 150 db the marked injuries moved in the direction of the basal portion of the first turn. Mild to moderate injuries were similar in apical extent for the two groups. Prolonged post-exposure life did not increase the extent of injury to the organ of Corti. Recovery of some of the mildly injured cells was apparent. Degenerative changes became more pronounced for hair and supporting cells that were moderately or severely injured by the exposure.

[1] Supported by United States Air Force Contracts in cooperation with the Bio-Acoustic Unit, Aero-Medical Laboratory, Wright Air Development Center.

D 27. Comparative uterine histology of two species of the mammalian order Lagomorpha.[1] Kenneth L. DUKE, Department of Anatomy, Duke University School of Medicine.

The species described are: *Ochotona princeps* of the family *Ochotonidae*, and *Lepus californicus* of the family *Leporidae*.

The uterine lumen of *Lepus* is +-shaped due to the presence of 4 major endometrial folds. Glands are numerous, even in the inactive uterus. The lumen of

Ochotona is roughly Y-shaped due to the presence of one major endometrial fold (mesometrial) and 10 or more minor ones. No glands are present but as estrus is approached the number of crypts increases.

The endometrium of prepubertal *Lepus* is 300 μ thick, that of *Ochotona* 98 μ. The myometrium of *Lepus* is 90 μ in thickness, that of *Ochotona* 102 μ.

Preimplantation endometria of *Lepus* are 1900 μ in thickness, those of *Ochotona* 425 μ. The uterine epithelium in *Lepus* measures 35 μ, that of *Ochotona* 25 μ. The myometrium of *Lepus* thickens to 800 μ, while in *Ochotona* to 925 μ.

Lepus develops a metrial gland in the intermuscular connective tissue; many giant cells (up to 1200 μ) develop in the endometrium. *Ochotona* develops no metrial gland, but the cellular walls of a bursa in the intermuscular connective tissue, at the mesometrial zone, undergo cyclic changes during pregnancy. Giant cells are fewer and much smaller than in *Lepus*.

[1] Aided by a grant from the Duke University Research Fund.

D 21. The architecture of the human adrenal cortex.[1] Hans ELIAS and John E. PAULY*, Department of Anatomy, The Chicago Medical School.

Stroma: The capsule consists of argyrophile fibers coated with collagen. In the zona glomerulosa, collagen membranes are suspended in the meshes of a reticular fiber network. This tissue forms sheaths around cords in the fasciculata. In the reticularis, a diffused fibrinoid-like tissue exists containing membranous condensations.

Vessels: The subcapsular, arterial plexus gives rise to capillary baskets in the glomerulosa, widening to large sinusoids in the fasciculata and reticularis.

Parenchyma: The following types of cell groups exist in the glomerulosa: club shaped cords, irregular cell masses, active glomerular follicles, atrophic glomerular follicles and syncytial bodies with inverted lipoid distribution. In every human adrenal gland, at least four of these five types of cell groups occur. Their distribution depends on the health of the individual.

All these groups of cells are connected with each other. The cortex is one parenchymal continuum.

The fasciculata consists of cords and plates connected by bridges.

The reticularis is a network of cell cords.

[1] Supported by grant H-1134 from U. S. Public Health Service.

D 56. Cytological observations on liver with the electron microscope. Don W. FAWCETT,[1] The Rockefeller Institute for Medical Research.

Rat liver was examined with the electron microscope with particular interest in the mode of attachment of the cells, the nature of their free surface, and the form and internal structure of their mitochondria. Cell surfaces contiguous with other liver cells have numerous stud-like processes which fit exactly into concavities of

appropriate shape in the opposing cell. These processes are often expanded at their tip while the concavities which receive them are constricted around their base. The interlocking structure which results closely resembles a mortise and tenon, and is interpreted as a device for maintaining attachment of adjacent cells. Cell surfaces which face sinusoids possess delicate filiform processes which are believed greatly to increase the surface available for interchange of material between the bloodstream and the interior of the cell. The mitochondria have two distinct membranes. The outer is a smooth continuous limiting membrane while the inner is thrown into folds and slender villi projecting inward. Mitochondria are observed occasionally which appear bisected by a transverse partition, comprised of two closely approximated membranes each continuous at its margin with the inner mitochondrial membrane. The interior of the mitochondrion is thus separated into discrete halves each limited by its own membrane but both contained within a common outer mitochondrial membrane. This double condition is tentatively interpreted as a stage in mitochondrial division.

[1] Present address: Department of Anatomy, Harvard Medical School, Boston, Mass.

D 48. Intravenous methylene blue for staining nerve fibers and endings. William FEINDEL*, Department of Neurology and Neurosurgery, McGill University, and The Montreal Neurological Institute. (Introduced by F. L. McNaughton)

Photomicrographs of various tissues will be shown to illustrate the use of intravenous methylene blue for neurohistological studies in rabbits and monkeys.

D 45. Connections of the Golgi cells and the intermediate cells of Lugaro in the cerebellar cortex of the monkey.[1] Clement A. FOX and Ewart G. BERTRAM*, Department of Anatomy, Marquette University School of Medicine.

The connections of the large cells (Golgi cells and intermediate cells of Lugaro) in the granular layer of the cerebellar cortex are demonstrated with photomicrographs of Golgi preparations.

Two types of Golgi cells are distinguishable: (1) superficial cells, located near the Purkinje cell layer, in which it is difficult to distinguish a single process generating the rich axonal plexus and, (2) deep cells, in which it is easy to distinguish a single process generating the axonal plexus. As is well known, the axons of Golgi cells contact the digits of granule cells.

The Golgi cells are contacted by the parallel fibers, recurrent collaterals of Purkinje cells and probably the axons of the basket cells.

A displaced Golgi cell and recurrent collaterals of Purkinje cells showing various patterns of branching are also demonstrated.

The intermediate cells of Lugaro are long, fusiform, horizontal cells located just beneath the Purkinje cell layer. Their dendrites and cell bodies are directed transversely in the folia and they are contacted by the "paint-brush" tips of the basket

cell axons, Golgi cell axons and short extensions from the mossy fiber rosettes. The axons of these cells ascend obliquely into the molecular layer where they divide several times, giving off longitudinal branches in the molecular layer. These branches run with and resemble the parallel fibers.

[1] Aided by a grant from the National Institutes of Health, Public Health Service.

D 16. Relation of the branching systems at the hili of a fused kidney. Carrie C. GILLASPY*, Department of Anatomy, State University of Iowa. (Introduced by W. R. Ingram)

The fused fixed kidney weighs 342.08 gm, measures 18.5 cm in its longest dimension, diameter at the middle of upper hilus is 5.5 cm, and at the middle of the lower hilus 9.5 cm. The anterior surface is slightly irregular with suggestions of lobulations in contrast to the posterior surface which is smooth.

The hili of the renal mass face anteriorly. Each renal sinus is expanded and shallow. The lower pelvis is almost replaced by two long major calyces which lie mostly outside the sinus. The inferior calyx of the superior hilus is broader and shorter than the superior one. The superior calyx passes anterior to the inferior one which it joins outside the sinus.

The hili, roughly triangular, are each almost completely surrounded by a vascular ring. It is formed by anastomoses of the branches of one (at upper hilus) or two (at lower hilus) arteries which enter either through the hilus or through the substance of the kidney. The superior vein has four main tributaries, the lower one drains from the inferior hilus, courses along its upper border, the other three drain the superior hilus and lie in relation to its medial surface. The inferior vein has four tributaries which course along the medial border of the inferior hilus.

D 2. Plastic-embedded sagittal sections of a newborn infant. Robert V. GREGG* and Charles F. BRIDGMAN*, Department of Anatomy, School of Medicine, University of California at Los Angeles and the Department of Medical Illustration, Veterans Administration Hospital, Long Beach. (Introduced by Charles H. Sawyer)

Continuing with the preparation of a teaching collection of anatomical specimens embedded in transparent plastic, we have employed a method modified from that of Kerns (Anat. Rec., *117:* 345, 1953) to demonstrate morphological relationships in a newborn infant. After attempting to drive the blood from the vascular system with a small amount of formalin, the specimen was injected with colored latex. It was then frozen in a block of ice to facilitate uniform sectioning with a thin-bladed band saw. Nine one-half inch sagittal sections were made through the head and trunk. Following sectioning the slices were allowed to thaw in Jores no. 1 fixative (10% glycerine added) and were subsequently kept refrigerated throughout dehydration. Our method differed from that of Kerns in the following details: eliminating the preparatory solutions other than Jores no. 1 to save time and color, refrigerating for further retention of color and mixing several monomers of the Selectron series to improve transparency of plastic and clarity of structural detail. Representative blocks of the series, with photographically reproduced labels and leaders, will be exhibited.

D 23. Observations on the number, size and structure of rat glomerular capillaries. B. Vincent HALL, Department of Zoology, University of Illinois, and Division of Biological and Medical Research, Argonne National Laboratory.

Enlarged transparencies made from 2 μ serial sections and electromicrographs (some serial) reveal that the apparent many small diameter glomerular capillaries are in reality openings cut into the walls of much larger, pouched and folded capillaries which when expanded probably have a relatively uniform diameter of about 15–20 μ. Individual capillaries may be traced from the entering afferent arteriolar tuft to a common exit basin leading to the efferent arteriole. Only intracapillary and extracapillary space and endothelial and epithelial cells (podocytes) have been found associated with glomerular capillaries. The ratio of endothelial to podocyte (epithelial) nuclear count is about 3:1. The relatively thick, homogeneous structure revealed by the light microscope, the basement membrane of the glomerular capillary, appears in electromicrophotography as a complex of three intimately related specialized structures (1) the luminal delicate highly porous submicroscopic layer, the lamina fenestrata (''lining network''), (2) a thin, smooth, dense but probably finely porous membrane, which must serve as an ultrafilter, the lamina densa, and (3) an intricate external layer, composed largely of characteristically shaped, rather uniform processes, the pedicels (''foot processes''), which are terminal extensions of secondary processes, trabeculae, from podocyte (epithelial) cytoplasm.

D 44. Demyelination in the central nervous system of genetically controlled mice and rabbits.[1] Pinckney J. HARMAN, Department of Anatomy, New York University College of Medicine and the Roscoe B. Jackson Memorial Laboratory.

Mutations occurring in inbred strains of animals may be preserved by controlled breeding and thus provide a continuous supply of study material. Histological preparations from brains and spinal cords of mutants with motor disturbances are demonstrated. Marchi, Nissl, Weigert and Bodian preparations of Dickie's ''Wabbler-lethal'' mouse and Sawin's ''ataxia'' in the rabbit reveal lesions in which abnormal myelin is a prominent feature. The lesions represent a reasonable explanation for the observed behavior abnormalities since archicerebellar and paleo-cerebellar systems are markedly involved. Mutants such as those of the present study should make possible an exhaustive analysis of the process of demyelination in experimental animal material.

[1] Supported by grants from National Multiple Sclerosis Society, U. S. Public Health Service, National Foundation for Infantile Paralysis, Office of Naval Research, Playtex Park Research Institute, The Anna Fuller Fund, The Jane Coffin Childs Memorial Fund for Medical Research and The National Advisory Cancer Council.

D 1. Monozygotic twin human embryos with an estimated ovulation age of 17 days. Chester H. HEUSER, Department of Microscopic Anatomy, Medical College of Georgia.

As seen in stereophotographs made during the dissection of the implanted chorion the embryos are normally attached to the mesodermal lining at points widely

separated from each other. Photomicrographs of the 6 μ sections reveal the axial structures cut favorably in the sagittal plane. Plastic sheet reconstructions made from the serial sections at magnifications of 200 diameters are also presented to portray the form and structure of each embryo.

D 15. The vascularization of the normal renal pelvis. W. Henry HOLLINSHEAD, Section of Anatomy, Mayo Clinic and Mayo Foundation, and Elmer DOUVILLE*,[1] Mayo Foundation, University of Minnesota.

The success of various plastic operative procedures upon the renal pelvis depends in part on an adequate blood supply, but descriptions of neither the normal vascular pattern nor the pattern in pathologically dilated pelves could be found in the literature. In the present investigation the renal arteries, and in a few instances the renal veins, of apparently normal kidneys were injected with latex or gelatin-india ink, the pelvis, calyces and upper part of the ureter were dissected free of fat and renal tissue, and the vascular pattern studied in opaque specimens and in specimens cleared and embedded in plastic. This study indicates that the normal renal pelvis and the major calyces receive directly small arteries derived from the renal artery and its branches both within and outside the renal sinus; that these anastomose freely with each other in the connective-tissue coat of the pelvis; and that they also anastomose with ureteric vessels and with numerous small arteries, derived from the interlobar arteries, that run downward along the minor calyces. The degree of anastomosis is such that no prevailing direction of blood flow, interruption of which might cause ischemia, could be determined. From the coarse anastomotic network finer channels are given off to the muscle layers and the submucosa.

[1] Fellow in Surgery.

D 19. Vertebrate chromosomes in tissue culture. T. C. HSU*, Department of Anatomy, Tissue Culture Laboratory, University of Texas Medical Branch, Galveston. (Introduced by John G. Sinclair)

By means of Ektachrome transparencies and actual microscopic slide demonstrations results obtained with the use of a hypotonic solution prefixation treatment for cells in tissue culture will be shown. Examples will illustrate normal chick, mouse, rat, dog, and human chromosomes. Abnormalities in human neoplasms will also be demonstrated.

D 14. Practical anatomy of the sphenomandibular ligament in relation to inferior alveolar nerve block. John E. HUGHES* and Niels B. JORGENSEN*, Department of Anatomy, School of Medicine and Department of Anesthesiology, School of Dentistry, College of Medical Evangelists. (Introduced by Otto F. Kampmeier)

Skull, wet specimen and illustration to show the attachments, extent and relations of the sphenomandibular ligament. Demonstrates its importance as a barrier in the inferior alveolar nerve block.

D 50. The blood vessels of the rat's eye.[1] Ralph G. JANES and George W. BOUNDS, Jr.*, Departments of Anatomy and Ophthalmology, State University of Iowa.

The blood vessels of the rat's eye have been injected with neoprene latex. Flat mounts have been prepared of the retina, iris and ciliary body, cornea and limbus and the choroid. Usually six arteries and six veins are found in the retina. These alternate as they radiate from the optic disc and give off numerous branches which end in a capillary network. The choroid receives its blood supply from the following ciliary arteries: small ones entering the eye in the region of the optic nerve, a larger one entering the sclera near the posterior pole of the eye which usually divides into three branches, and finally branches entering the choroid from the two long posterior ciliary arteries. The latter arteries form the major arterial circle of the iris and pierce the sclera near the equator of the eyeball. Anterior ciliary arteries are not clearly defined but generally consist of two small vessels which form the arterial circle of the limbus (arterial circle of the corneo-scleral limbus). Anastomoses occur between the arterial circles in the region where the long posterior ciliary arteries enter the iris. Most of the blood is drained from the eye by the vortex veins. These vary in number and usually leave the eyeball just posterior to the equator.

[1] Aided by a grant from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

D 4. Studies on the developmental morphology of the connective tissue and the vascularization of the human tongue. Ekkehard KLEISS*, Department of Anatomy, Universidad de Los Andes, Mérida, Venezuela. (Introduced by O. P. Jones)

In serial sections of the tongues of human embryos and fetuses between 16 mm and full term, and of 14 children and adults (till 45 years) the morphogenesis of the connective tissue was investigated. Furthermore, diaphanized gross sections and histological preparations of the tongues of totally injected fetuses between 14 and 35 cm, and injected tongues of children and adults were used for the studies of the vascular system of this organ.

The development of structural details of the different parts of the connective tissue of the tongue (submucous fibrous stratum and septum) is described and compared with their definitive morphology, concerning especially their functional significance. In the same way, the morphogenesis of the blood vessels is studied and there are reported some new findings on the vascularization of the papillae. The important paper of blood vessels and connective tissue in the development of papillae is stressed, in order to give an hypothetical idea of the functional correlation of different tissues during development.

D 18. Demonstration of terminal air passages and alveoli in fresh lungs of newborn mice.[1] Vernon E. KRAHL, Department of Anatomy, School of Medicine, University of Maryland.

Fresh lungs of newborn mice are placed in a small transparent chamber where they are slowly inflated by exposure to subatmospheric pressures. Air entering the

lungs at atmospheric pressure progressively expands bronchioles, respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli in peripheral, atelectatic areas of the lungs. The method permits a simple classroom demonstration of normal shapes and relationships of these structures. The spaces may be measured, counted, et cetera without employing the more tedious technique of wax plate reconstruction. When such lungs are filled with Ringer-Locke solution, higher magnification may be used for the observation of the finer details of alveolar structure and of cellular and noncellular contents of alveoli.

[1] Supported by a grant from the American Trudeau Society.

D 25. *The occurrence of tubulo-acinar glands of the eustachian tubes in adult mice.* Aaron J. LADMAN*[1] and Arthur J. MITCHELL*, Department of Anatomy, Harvard Medical School. (Introduced by George B. Wislocki)

In adult male mice, compound tubulo-acinar glands forming diverticula of the eustachian tube are found in the carotid canal medial to the tympanic bullae in the region of the articulation of the basisphenoid and occipital bones. The bilaterally situated glands have topographical relations with the dura mater of the hypophysis, the carotid artery and cranial nerves V and VI. In newborn mice, the region in which these glands later develop, comprises a thick layer of connective tissue. As this region grows and differentiates, the connective tissue layer becomes thinned out so that these glands assume the relationships mentioned above.

The terminal portions of the glands have the histological appearance of serous acini. However, the apices of the cells contain granules which stain with the periodic acid-Schiff method after diastase digestion, a result which indicates the presence of a "mucoid" substance. From these observations it appears that the cells are of the "seromucinous" variety.

The gland ducts, several of which empty close together into the eustachian tube, are lined by tall columnar mucous cells. After diastase digestion, these cells exhibit an intense periodic acid-Schiff reaction and lack basal striations. Thus, these ducts resemble the intralobular ducts described in submaxillary glands of male mice (Fekete, '41).

[1] Fellow of the American Cancer Society, Inc., 1952–54.

D 18. *In vitro experiments on the effect of intact mouse sarcomas and cell-free homogenates on sensory and sympathetic ganglia of chick embryo.* Rita LEVI-MONTALCINI, Stanley COHEN* and Herta MEYER*, Department of Zoology, Washington University, and Instituto de Biofisica, University of Brasil.

Small pieces of sarcomas 37, 180 and 1 were explanted in vitro in proximity to sensory or sympathetic ganglia of 6–13 day chick embryos. After 24 hours, ganglia facing the tumor had produced a dense "halo" of nerve fibers whose density was maximal on the side toward the tumor. On this side the fibers were shorter than on the other sides. In contrast to control cultures they grew in a straight radial direction. The tumor effect was maximal with sarcomas 37

and 180 and milder with sarcoma 1. No effect was detected with mammary adenocarcinoma C3H and with neuroblastoma. An inhibition of fibroblast growth was observed concomitantly with the increase in nerve fibers. Control cultures in which the same ganglia were combined with normal chick tissue showed after 24 hours only few nerve fibers but a large number of migrating fibroblasts. Controls in which the ganglia were combined with embryonic mouse tissue showed a more pronounced nerve growth but not comparable with the outgrowth called forth by the sarcomas.

Sensory and sympathetic ganglia growing in a standard medium to which cell-free homogenates of these tumors were added, showed an increase in nerve fibers comparable to that produced by the intact tumors. The microsome fraction isolated from these homogenates contained most of the nerve growth-promoting material. The effect was clearly evident in 12 hours.

D 34. The "impression technic" in histochemical cytological investigations.[1] John F. LHOTKA and Louis TURLEY*, Departments of Anatomy and Pathology, The University of Oklahoma School of Medicine.

The "impression technic," a highly neglected method, has shown much promise as a histochemical cytological procedure. It has been used satisfactorily with practically all of the microscopical histochemical technics such as the PAS method, the Feulgen reaction, the Kurnick stain, and a number of the enzyme technics which do not require special treatment in fixation or processing. Impressions are made simply by pressing a freshly unfixed section of the organ to be studied briefly against a clean slide, either albuminized or not, and then immersing the slide in the appropriate fixative. The slide is then processed according to technic as if it were a smear or section. In some cases the slide may be air-dried prior to fixation or even submitted to the histochemical procedure without fixation. Cellular distortion with this method has been minimal in comparison to that obtained with the "smear" technic now in favour. Although this method has been used spasmodically for many years, it was felt that it would be of value to call it to the attention of histologists as an extremely satisfactory method for preparing tissues for histochemical examinations of individual cells. In addition, it is an excellent method for preparing tissue for certain routine pathological studies such as investigating lymphoid tissues for Reed-Sternberg cells or cerebrum for Negri bodies.

[1] Supported by a grant from the Biological Stain Commission.

D 35. Ultrastructure of the pulmonary alveolar wall. Frank N. LOW, Department of Anatomy, Louisiana State University School of Medicine.

Selected electron micrographs of sectioned lung tissue fixed by the Palade technique for a variable number of hours are used to illustrate ultrastructure in the pulmonary alveolar wall. Unit fibers of collagen are conspicuous. They may be loosely arranged in patterns invisible to the light microscope or be thickly aligned longitudinally to form the reticular fibers of light microscopy. Elastic tissue is present both as fibers and as fenestrated sheets. A thin basement membrane

lines both capillary endothelium and alveolar epithelium. Heavily osmiophilic, these membranes often appear to be homogeneous. But, within them in fortuitous preparations, fine fibrillar material of smaller diameter than unit fibers of collagen may be observed. These membranes are indistinguishable from each other except by position and appear to constitute the main structural strength of the alveolar walls.

D 24. Outlets for alveolar fluid from air spaces into lymph vessels, and dust traps associated with them, in mammalian lungs. Charles C. MACKLIN, Department of Histological Research, University of Western Ontario.

Sections from adult canine, human and other dust-exposed lungs show varisized, often prominent, dark masses of dust particles, mainly in the stems of the primary lobules. Many are crescentic and hug the outer borders of myoelastic rings of transitional and respiratory bronchioles. Much dust is in reticuloendothelial cells between a plaque of modified epithelium in the wall of the air-tract (perhaps the stoma of Sikorsky, 1870) and the local definitive lymph capillary. An explanation of its presence here is that it has evaded the phagocytic pneumonocytes which ordinarily would have removed it via the glottis, and has drifted in with the overflow of aqueous mucoid fluid. In morphology these dust traps recall sinuses of lymph nodes. Movements of the muscle of Reisseissen may promote the flow of lymph within this channel. The usually smaller dust masses of like morphology and relations occurring near the pulmonary venules and arterioles are similarly interpreted; as are also the flattened accumulations in the pleura interpolated between outlets in the walls of subpleural alveoli and the local lymph vessels. That these various dust arrestors are not completely efficient is evident from the finding of dust in hilar lymph nodes and even systemic nodes. When viable bacteria or tumor cells are thus incarcerated a nidus of disease is formed.

D 51. The autonomic nervous system: Reels 3 to 6. J. E. MARKEE, R. F. BECKER, Maude F. WILLIAMS*, George LYNCH*, A. C. WEBSTER* and A. Lee BARRETT*, Duke University.

The demonstration consists of twenty-four 8 × 10 inch ectachrome transparencies which illustrate the key scenes in the motion picture on the autonomic nervous system reels 3 to 6.

In the motion picture is shown the autonomic innervation of the adrenal gland in reel 3: of the bladder in reel 4: of the eye in reel 5 and of the salivary glands in reel 6. In the motion picture a simplified animation technique enables the viewer to follow the course of the nerve fibers from the central neuraxis to their peripheral destination. In the demonstration, the completed diagrams are shown with appropriate labels.

D 42. A stereotaxic instrument designed for use on rats.[1] W. R. McCRUM and L. C. MASSOPUST, Jr.*, Department of Anatomy, University of Colorado.

Two devices for placing lesions in brains of rats have been combined in a single unit. Using only base and head holder horizontal electrodes can be introduced into the hypothalamus through a trephine opening in the temporal region. Accurate positioning of an electrode whose uninsulated part bridges the third ventricle facilitates the production of bilateral lesions with a single application of current. Reproducible, moderate-sized, lesions can be made rapidly in a series of animals of uniform size.

To provide for more minute localization of both bilateral and unilateral lesions, a conventional stereotaxic mechanism may be attached to the base in such a manner as to utilize the same head holder as is used for the temporal approach.

[1] Supported by grants from Armour and Company and the United States Public Health Service.

D 17. The distribution of blood vessels and nerves in the temporomandibular joint of the Rhesus monkey.[1] Benjamin C. MOFFETT, Jr.*, Department of Anatomy, Medical College and School of Dentistry, University of Alabama. (Introduced by James O. Foley)

Intravascular injections of india ink in Rhesus monkeys show an arterial ring, derived from the internal maxillary artery, surrounding the neck of the condyle. Branches from this ring, from the superficial temporal artery and from arteries in the mandibular periosteum run vertically in the joint capsule to form a synovial plexus, especially prominent on the posterior portion of the articular disc. Communications between these vertical branches form another vascular ring in the lateral and medial recesses of the inferior joint cavity. In addition to the veins which accompany all these vessels, other veins drain anteriorly into the pterygoid venous plexus. The compressed portion of the disc contains no blood vessels.

Sections of the articular disc stained for nerves by the Bodian and urea silver nitrate techniques show myelinated and nonmyelinated nerve fibers, most plentiful in the posterior part of the disc. These are probably from the auriculotemporal nerve and terminate as free or encapsulated endings in the subsynovial tissue facing the inferior joint cavity. Occasionally fibers are seen extending into the synovial tissue or into a synovial villus. Nerve fibers entering the anterior attachment of the disc and presumably from the masseteric nerves are scarce. No fibers are seen in the central compressed portion of the disc.

[1] The blood supply was investigated in the Department of Anatomy, College of Dentistry, New York University.

D 6. A new type of placentation demonstrating the phylogenetic and taxonomic significance of the fetal membranes.[1] H. W. MOSSMAN and C. H. CONAWAY*, Department of Anatomy, University of Wisconsin.

The African jerboa, *Jaculus*, and the North American jumping mouse, *Zapus*, have a placenta best described as of the "hemochorial, giant-cell type." Mononuclear trophoblastic giant-cells, always confined to the decidual or outer surface

of the labyrinth in other known forms, here at an early stage seem to penetrate through perforations in the cytotrophoblast of the true chorion and invade the mesoderm of the allantoic bud when it contacts the chorion. They sheathe the allantoic vessels as a single layer. Having themselves formed channels carrying the maternal blood stream through the perforations into the allantois, they thus establish a hemochorial placentation in the allantoic mesoderm, on the fetal side of the zone where such a placentation is usually formed by invasion of mesodermal allantoic villi into the chorionic syncytium. The discovery of this unusual placentation in both the Dipodidae and Zapodidae indicates close relationship between the two groups, and lack of close relationship between them and the suborder Myomorpha in which they are usually, but hesitantly, placed. This is another clear-cut demonstration of the value of the fetal membranes as criteria for phylogenetic relationships.

[1] Supported by grants from the Wisconsin Alumni Research Foundation. The Jaculus material was examined through the courtesy of the Hubrecht Laboratory, Utrecht, Netherlands.

D 46. A silver impregnation method for staining Muller's fibers in the retina after paraffin imbedding. T. K. NASSAR* and W. M. SHANKLIN, Department of Histology, American University of Beirut Medical School, and Department of Anatomy, Medical College of Virginia.

Pieces of retina are fixed in 10% formalin 5 days at room temperature and are washed twice in distilled water, to which are added 10 drops concentrated ammonia per 100 ml., followed by 2 changes in distilled water. Tissues are dehydrated in alcohol, cleared by cedar oil, infiltrated and imbedded in paraffin and sectioned. Sections are hydrated and placed in 10% silver nitrate, to which is added 3 drops pyridine per 100 ml., for 24 hours at room temperature and washed in distilled water, to which is added 2 drops pyridine per 100 ml. Sections are next impregnated in silver diamminohydroxide, to which is added 3 drops pyridine per 100 ml., in the incubator at 50°C. for 3 to 6 minutes. They are then dipped in distilled water and reduced in 2% formalin, to which is added 8 drops pyridine per 100 ml., for 5 to 10 minutes. The sections are washed in water, toned in gold chloride, fixed in hypo, washed, dehydrated, cleared and mounted in balsam. Branches of Muller's fibers, especially in layer 9, are beautifully stained by this method.

D 32. A small particulate component of the cytoplasm. George E. PALADE, The Rockefeller Institute for Medical Research.

A granular component of small size and relatively high electron density has been regularly encountered in the cytoplasm of various cell types examined in the electron microscope. The particles are usually spherical, rarely rod-like, and measure 80–300 Å in diameter, dimensions usually ascribed to macromolecules.

In many adult cells (liver, exocrine glands) they are associated with certain vesicular and tubular elements of the endoplasmic reticulum, being attached to the outer surface of their limiting membrane, on which they frequently form orderly patterns. Fewer granules, isolated or in clusters, are found scattered in the surrounding cytoplasm. The second (cytoplasmic) nuclear membrane is

frequently covered with similar granules, whereas the cell- and mitochondrial membranes are free of them.

In embryonic and rapidly multiplying adult cells (lymphoblasts, epithelia of the intestinal crypts, basal layer of the epidermis) numerous granules are scattered throughout the cytoplasm with only a few attached to the elements of the endoplasmic reticulum.

The granular component is particularly abundant in the cells with an intensely basophil cytoplasm (liver cell, plasmacells, acinary cells of salivary, mammary glands and pancreas, lymphoblasts, angioblasts, etc.). This finding together with results obtained in cell fractionation studies suggests that the new component may be partially responsible for the cytoplasmic basophilia and may correspond to the ultramicrosomes described by certain cytochemists.

D 38. Electron microscopy of marrow cells.[1] Daniel C. PEASE, Department of Anatomy, School of Medicine, University of California at Los Angeles and Sawtelle Veterans Administration Hospital.

Red marrow of rats is being studied by means of ultrathin sections and electron microscopy. Micrographs representing the major cell types will be demonstrated. One of these cells is characterized by a rich development of the endoplasmic reticular system. Serial sections prove it to be in the form of anastomosing plates in this instance. White thrombi will also be demonstrated, and the structure of platelets compared with megakaryocyte cytoplasm.

[1] Aided by the Los Angeles Heart Association and the U. S. Public Health Service.

D 39. Observations on the submicroscopic structure of animal epidermis. Keith R. PORTER, The Rockefeller Institute for Medical Research.

An examination of the epidermis of several vertebrates, but most especially of larval forms of Amblystoma, has defined the nature and disposition of keratin in the component cells and described the structure of intercellular bridges and the basement membrane. Keratin is present in the fine filaments, $\sim$ 60 Å thick, organized in dense skeins around channels of non-fibrous elements of the cytoplasm. The skeins are localized mostly in the cortical regions of the cells and are oriented parallel to the epidermal surface. The "bridges" appear as small regions of contact shared by contiguous cells. They are characterized by (a) an apparent thickening of the membrane of both cells, (b) by tufts of fibrous material directed from the membranes toward the cell interior, and (c) by the presence of striae across the tufts. There is no evident continuity of filaments or cytoplasm across the bridges. The epidermal cells rest on a basement membrane consisting of (a) a layer of granules subjacent to the plasma membrane, (b) under this a layer of unorganized material blending into (c) a fabric of fine fibrils. These fibrils are $\sim$ 130 Å in diameter and in some instances they appear striated at intervals of 350–400 Å. They are arranged in several layers parallel to the epidermal surface and, in successive layers, are oriented at right angles. With some variation these same features of fine structure are evident in the epidermis of the rat and other vertebrates.

D 5. Circulation in the maternal placenta. Elizabeth M. RAMSEY, Department of Embryology, Carnegie Institution of Washington.

One of the central problems of placental circulation may be expressed as follows: If arterial and venous openings are distributed indiscriminately over the base of the placenta, how can circulation throughout the intervillous space (IVS) be effected? Why does not entering arterial blood "short-cut" at once to neighboring venous exits?

The classical solutions of Bumm (1892) and Spanner ('35) are in part contradicted by recent observations in 15 human placentas of various stages (8 weeks to term) injected *in situ* with India ink and studied in serial sections. Thus, contradicting Bumm, arterial entries do not characteristically occur high on the maternal septa but in the basal plate and, contrary to Spanner's contention, basal veins are demonstrable all over the placenta, at all stages of pregnancy, and drainage is not exclusively marginal.

Photographs and microscopic preparations demonstrating this new evidence will be exhibited and the conclusion supported that separation of afferent and efferent blood is primarily effected by pressure differentials. Maternal arterial blood entering the IVS under pressure 60–70 mm. Hg higher than that within the IVS is driven toward the subchorial lake. At the same time the contents of the IVS converge upon venous exits because, between contractions, IVS pressure exceeds that within the maternal veins. Morphological arrangements assist but do not occasion intervillous circulation.

D 3. Studies on the development of the gingivo-labial connective tissue in man. Luis RENGEL SANCHEZ*, Department of Anatomy, Universidad de Los Andes, Mérida, Venezuela. (Introduced by N. L. Hoerr)

The material for this investigation were serial sections of the lower jaw and tongue of human embryos and fetuses between 19.5 mm and 35 cm, stained with Haematoxylin-Mallory or Azocarmine-Mallory or Orcein. Injected specimens (fetuses) were used too.

The fibrillar structures are described in different developmental stages, especially in the sublingual connective tissue and in the gum and lip. In the latter, the development of the subepithelial venous plexus and its functional significance in the new born infant are discussed. The developmental topography of the elements in the sublingual connective tissue is described and its connections with the tongue (especially in a part which can be considered as the "hilum of the tongue") is stressed. In the structural aspects of the gum, the embryologic findings are compared with the definitive situation of fibers as they are present in adult preparations. Finally, there is given clear evidence on the development of the anterior part (mental region) of the mandible from Meckel's cartilage, which can be observed already in rather early stages (about 50 mm).

D 26. Histochemical demonstration of the locus of mercurial inhibition of succinic dehydrogenase in the rat kidney. Edward G. RENNELS and Arthur RUSKIN*, Departments of Anatomy and Internal Medicine, University of Texas Medical Branch, Galveston.

Incubation of fresh-frozen sections of kidney in a buffered medium containing neotetrazolium and sodium succinate results in reduction of the neotetrazolium to a colored formazan, which is deposited at the sites of succinic dehydrogenase activity. In normal rat kidneys this method reveals dark coloration in the convoluted tubules of the cortex, whereas the glomeruli are essentially uncolored. The medulla exhibits three distinct zones; the innermost of these occupies approximately one-half of the medullary area and is only feebly colored due to its content of unstained collecting ducts and poorly stained, thin segments of Henle's loops. The intermediate zone is intensely colored due to the concentration of very reactive, ascending (thick) limbs of Henle's loops in this area. The outer medullary zone is somewhat less intensely colored than the intermediate since the descending limbs of Henle's loops concentrated in this region are less reactive.

When mercuhydrin is administered to rats by subcutaneous injection there is a marked depression of the succinic dehydrogenase activity in the proximal convoluted portion of the kidney tubules, while other portions of the nephron are little affected. This enzymatic inhibition is concomitant with the known time of diuresis and both can be prevented by the administration of BAL. This places the locus of mercurial action on the proximal convoluted tubule.

D 9. Lens induction in Amblystoma punctatum.[1] Randall W. REYER, Department of Anatomy, University of Pittsburgh.

Lens induction in *Amblystoma punctatum* was studied in 71 cases where the prospective lens-forming and adjacent head ectoderm from hosts in either early neurula or early head and tailbud stages was replaced by prospective belly ectoderm from middle gastrula, early neurula or early head and tailbud donors previously stained in Nile blue sulphate. In 15 additional cases, prospective lens ectoderm of an early head and tailbud host was replaced by prospective lens and adjacent head ectoderm from an early neurula donor. The vital stain was preserved in the sections following fixation 4 to 6 days after operation.

Induction of a lens or lentoid from prospective belly ectoderm occurred in grafts from gastrula donors to early neurula hosts (7 out of 15 cases) but in no other combination. The optic vesicle of a head and tailbud host induced a lens from neurula prospective head ectoderm only (13 out of 15 cases). Self-differentiation of prospective lens ectoderm grafted to the belly was observed with head and tailbud donors but not as yet with grafts from early neurulae.

These results confirm previous work and also provide additional evidence for the action, in this species, of two lens inductors, first, the head mesoderm and second, the optic vesicle. This diphasic induction resembles that found in ear development.

[1] Supported by grants from the U. S. Public Health Service.

D 11. Induced and spontaneous tumors in tumor-susceptible guinea pigs.[1] James B. ROGERS and Richard C. TAYLOR*, Departments of Anatomy and Pathology, University of Louisville School of Medicine.

Guinea pigs, descendants from ancestors which had spontaneous tumors, have been tested for tumor development by (1) aging to senility and (2) treatment with a chemical carcinogen, methylcholanthrene.

Survival past 1095 days (three years) is considered senility. Approximately 275 animals have reached senility. Of these, 47 are living. Of the 228 senile animals which have died, 15.5% were found to have spontaneous tumors of various kinds at post-mortem examination.

Guinea pigs of various ages, 42 to 1862 days, were treated with methylcholanthrene in sesame oil injected subcutaneously in the left subcostal region. One mg. of methylcholanthrene in 1 cc. of sesame oil repeated once after 30 days was the smallest amount used. Ten mg. of methylcholanthrene in 1 cc. of sesame oil repeated twice at 15 day intervals was the largest amount used.

Of 10 young animals treated with 1 mg. of methylcholanthrene repeated once, 50% developed palpable sarcomas after 499 days (average).

Of 30 young animals treated with 10 mg. of methylcholanthrene repeated twice at 15 day intervals, 70% developed palpable sarcomas after 184 days (average).

Of 17 senile animals treated with 10 mg. of methylcholanthrene repeated twice at 15 day intervals, 76% developed palpable sarcomas after 90 days (average).

[1] Aided by a grant from the U. S. Public Health Service and by a grant to the University of Louisville from the Kentucky State Medical Research Commission.

D 20. Anterior pituitary in tissue culture. B. ROSENBERG*, Department of Anatomy, Tissue Culture Laboratory, University of Texas Medical Branch, Galveston. (Introduced by C. M. Pomerat)

The tissue culture of the anterior pituitary of the rat, dog, and cat under various experimental conditions will be demonstrated by means of color transparencies, black and white photomicrographs and actual microscopic preparations. The effect upon the anterior pituitary of these species with the addition of various concentrations of hormones will be shown.

D 28. Histochemical study of the carotid body of the rat and cat. Leonard ROSS* and Thomas E. HUNT, Department of Anatomy, Medical College and School of Dentistry, University of Alabama.

The carotid body of the cat and rat has been examined histochemically for the occurrence of ribonucleic acid (RNA), alkaline phosphatase and periodic acid-Schiff (PAS) positive material. Cold 95% alcohol afforded better fixation for all procedures than either Carnoy's or cold 10% neutral formalin. In the carotid of the cat, RNA as demonstrated by methyl green-pyronin is present as discrete granulations sparsely scattered between numerous cytoplasmic vacuoles. In the rat, the granules of RNA are much more abundant. The presence of RNA is further confirmed by toluidine blue basophilia. In both animals ribonuclease abolished the pyroninophilic and basophilic reactions of the granules. In the cat, alkaline phosphatase (Ca-Co method) is concentrated in considerable amounts at the periphery of most cells and

nuclei but is not demonstrable in the blood vessels. In the rat, on the other hand, alkaline phosphatase is abundant in the vessels but is absent in relation to the chemoreceptor cells. In the carotid bodies of both the cat and rat, PAS positive material is concentrated at the periphery of the chemoreceptor cells but none is present within the cytoplasm.

D 53. Acetyl-cholinesterase in spinal ganglia of the dog. Mary Elmore SAUER, Department of Anatomy, The University of Texas, Medical Branch.

Many of the spinal ganglion cells of the dog apparently contain acetyl-cholinesterase in high concentration, while the remainder contain only a trace. With Couteaux and Taxi's modification of Koelle's method, staining is best in an incubation medium at pH 5.3–5.1. The deeply stained cells seem to be more uniform in size than the cells of the ganglion as a whole.

This picture for dog spinal ganglia is in striking contrast to the "extremely low concentrations" of acetyl-cholinesterase reported by Koelle for spinal ganglia of the cat.

D 41. The maturation of the hypothalamic-hypophyseal neurosecretory system in the dog.[1] Ernst SCHARRER, Department of Anatomy, University of Colorado School of Medicine.

The first appearance and gradual accumulation of neurosecretory material in the supraoptic and paraventricular cells, in the tractus supraoptico-hypophyseus, and in the neurohypophysis were studied in basset hounds and mongrel dogs from fetal to adult age. In fetuses, about one week before birth, only very few granules are found in the neurosecretory cells in sections stained with Gomori's chrome hematoxylin. However, since at that age the endings of the tractus supraoptico-hypophyseus in the posterior lobe contain considerable amounts of neurosecretory material, it must be assumed that submicroscopic aggregates are carried along the axons to the posterior lobe prior to the appearance of visible granules in the secreting nerve cells. At any given time throughout the pre- and postnatal development the posterior lobe shows more neurosecretory material than the secreting cells in the same animal. In young dogs, practically all the cells of the supraoptic and paraventricular nuclei contain moderate numbers of evenly distributed granules of fairly uniform size. The posterior lobe of young animals also offers a more uniform picture than that of the adult. With increasing age, the masses of neurosecretory material both in the hypothalamus and in the neurohypophysis become more irregular, Herring bodies appear, and the neurosecretory cells vary widely in the amount of granular content and some become grossly vacuolated.

[1] ONR contract No. 6onr-231-VIII.

D 22. Venous sinuses and arterial capillary terminations in the spleen of the gibbon. Theodore SNOOK, Department of Anatomy, University of North Dakota.

Sections of the spleens of two gibbons (Hylobates lar) were studied by the reticular fiber impregnation technique. In one of the specimens, the blood filling the

venous sinuses was uniformly blackened by a silver deposit and thus appeared as a nearly perfect vascular injection of the venous sinus system. In addition to this graphic representation of venous sinuses and their interconnections, arterial capillary terminations will be demonstrated.

D 39. The structure of neuroglial cells as observed with the electron microscope.[1] V. L. van BREEMEN, Department of Anatomy, University of Colorado.

Rat brain and spinal cord tissues were fixed in acetate-veronal buffered osmium tetroxide or in osmium tetroxide in Tyrode solution (pH 7.4–7.5). The tissue pieces were embedded in methacrylate, sectioned at ca. 0.05 μ and examined with a Philips electron microscope.

The neuroglial cells illustrated in the electron micrographs may be identified with the classical protoplasmic astrocytes. However, the typical protoplasmic projections were not found. Rather, the glial cytoplasm seems to be quite extensive and bounded finally by a cell membrane. Nerve fibers extend through the cytoplasm of the glial cell between its nucleus and outer membrane.

[1] Supported by a grant from the Muscular Dystrophy Associations of America.

D 43. Wallerian degeneration of the lateral olfactory tract. A. VAZ FERREIRA, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay.

Partial or total chirurgical resections of the olfactory bulb, and small electrolytic lesions placed at the various levels of the same, were performed in albino rats. After variable survival times, the resulting Wallerian degeneration of the lateral olfactory tract was examined by means of some of the modifications of Cajal and Bielschowsky (including Glees) methods, with the scope of allowing a comparative study among these. Golgi technique is used for demonstrating the normal aspects. Slides and photomicrographs form the basis of this demonstration.

D 30. Morphology and cytochemistry of the cell components of the thyroid gland. Emilia VICARI, Roscoe B. Jackson Memorial Laboratory.

The present observations were undertaken in an attempt to provide additional data on the nature of the parafollicular and interfollicular cells of the thyroid gland. Selective staining other than the silver impregnation has revealed results which may bring about a reconsideration of the nature and origin of these cells. Photomicrographs and microscopic slides will be shown.

D 37. Electronmicroscopic studies of the amphibian basement lamella.[1] Paul WEISS and Wayne FERRIS*, Department of Zoology, University of Chicago.

Ultrathin sections of larval skin (urodele and anuran), viewed electronmicroscopically, show the subepidermal basement lamella to consist of a regular fabric of cross-striated collagen-like fibrils embedded in ground substance. The fibrils, ca. 500 Å thick and with a period of (±) 520 Å, are arranged in (±) 20 strata, each containing strictly parallel fibers, about five deep (0.25 μ), in orientations that alternate regularly by 90° from layer to layer, like plywood. In frog embryos, this pattern is first discernible about Shumway stage 22. During its formation, the epidermal nuclei lie close (ca. 0.2–0.4 μ) to the basal surface. Later, the epidermal

cytoplasm shows a fine filamentous (cotton-like) texture, which upon removal of the methacrylate embedding medium breaks down into the coarse beaded reticulum often depicted in standard electromicrographs. The cytoplasmic filaments converge upon peculiar bobbin-shaped, heavily electron-absorbing bodies arrayed in a single layer just inside the cell surface. These bodies (measuring about 1000 ×1000 Å) are confined to the surface of the epidermis adjoining the basement lamella and may be concerned with either fibrogenesis or mechanics of attachment. Controlled modifications of these fine-structural details by experimental interventions are now being studied.

[1] Supported by grants from the Abbott Memorial Fund, University of Chicago; and the American Cancer Society (through the Committee on Growth, NRC).

D 8. The use of an improved vibrating needle for embryonic operation and dissection. Byron S. WENGER* and Cullen H. MACPHERSON*, Department of Anatomy, University of Kansas. (Introduced by Howard A. Matzke)

The vibrating needle described by Drury, 1941 (Science, *93,* 263) has been modified in two aspects. The first, reported previously (Wenger, 1951, J. E. Z., *116,* 123), involves bending the blade approximately 45° out of line with the shaft and in a plane perpendicular to the plane of vibration. The second modification affects a considerable increase in cutting efficiency replacing the 60 cycle vibration of ordinary alternating current with a vibration up to several thousand cycles per second provided by a variable audio frequency generator. A crystal phonograph recording cartridge replaces electro magnet of Drury's instrument for translating the oscillating current into mechanical vibration.

D 12. Malformations caused by azo dyes.[1] James G. WILSON, Department of Anatomy, University of Cincinnati.

Three azo dyes, azo blue, trypan blue, and Evans blue, caused congenital malformations in the offspring of female rats injected with 1-cc doses of a 1% aqueous solution on the 7th, 8th and 9th days of pregnancy. Although the percentage of abnormal offspring produced with each dye varied coniderably (59% with azo blue, 49% with trypan blue and 14% with Evans blue), the types of malformations were approximately the same in each case. No group of defects occurred in association with sufficient regularity to establish a syndrome, but the relative frequency of individual malformations was moderately constant.

Defects of some type were seen in virtually all organ systems, but the brain, eye, and vertebral column were by far the most common sites of maldevelopment. Such gross anomalies as exencephaly, meningocele, anophthalmia, gastroschisis and absent tail were easily recognized in the intact fetus or newborn. Skeletal defects involving fused or missing lumbar or sacral vertebrae, however, were best visualized in cleared alizarin-stained specimens. Several other anomalies such as ocular malformation, moderate degrees of hydrocephalus, and cardiovascular and genitourinary defects were detected only in dissected or serially sectioned animals.

Enlarged photographs as well as intact, cleared, and dissected specimens illustrating the types of anomalies will be displayed.

[1] Supported by a grant (A 105) from the National Institutes of Health, Public Health Service.

MOTION PICTURES

MP 4. Cytoplasmic activity in living spinal ganglion cells of the rat. F. B. ADAMSTONE, Department of Zoology, and A. B. TAYLOR*, Department of Physiology, University of Illinois.

Motion pictures are presented showing the activity of cytoplasm and cellular organelles in the living neurone of the rat. Pigment granules, mitochondria and other granules can be seen in motion as well as the undulations of a darker net-like reticulum interpreted as the Golgi net. Cessation of movement in response to the introduction of various chemical substances is also shown and this is regarded as further evidence that the cells are alive. The activity of the neurone is also recorded using phase contrast optics. These observations give a new concept of the neurone as a dynamic entity in contrast to the generally accepted idea of a static, semi-jelled structure.

MP 1. The autonomic nervous system: Reels 3 to 6. R. F. BECKER, J. E. MARKEE, Maude F. WILLIAMS*, George LYNCH*, A. C. WEBSTER* and A. Lee BARRETT*, Duke University.

This is a 16 mm sound, color, teaching film. The fundamental plan of all the reels is first demonstrated by means of colored diagrams. By means of a simplified animation technique the viewer may follow the course of the nerve fibers from the central neuraxis to their peripheral destination. By an appropriate choice of differentiating colors, the nature of the two divisions falls into easy contrast.

Interspersed with the diagrammatic material are selected dissection specimens for emphasis of the actual detail as it is encountered in the cadaver. For this showing, the sound is recorded on a magnetic stripe on the film.

The autonomic innervation of the adrenal is illustrated in reel 3: of the bladder in reel 4: of the eye in reel 5 and of the salivary glands in reel 6. An exhibit will show the key scenes in the motion picture by means of twenty-four 8 × 10 inch ectachrome transparencies.

MP 5. A cinematographic record of the transport of ova from the periovarial space into the oviduct of the rat. Richard J. BLANDAU and Anthony CANEDO*, Department of Anatomy, University of Washington.

The periovarial sacs of rats in heat were opened and the fimbriated ends of the oviducts exposed. The transport of supravitally stained ova over the surface of the fimbria of the oviduct and into the dilated loops of the ampulla was recorded cinematographically on 16 mm Commercial Kodachrome. Both ciliary and muscular action participate in transporting newly ovulated eggs into the oviduct.

MP 11. The structure and function of the vestibular apparatus. Newton B. EVERETT, Department of Anatomy, University of Washington. (Assisted by Richard J. Blandau)

This is a 16 mm, Kodachrome, sound, teaching film which presents the gross and microscopic anatomy of the human utricle, saccule and semicircular canals. The

way in which these structures respond to stimuli resulting from alterations of the body's orientation in space or to a change in deceleration or acceleration is demonstrated by means of animated drawings. The functions of these vestibular parts of the ear are further demonstrated by using the crayfish, frog and rat as experimental animals. Nystagmus is explained and demonstrated in a human subject after rotational and after caloric stimulation.

MP 8. Mechanism of petechial formation (hamster and frog).[1] George P. FULTON, Maurice H. SHULMAN*, Brenton R. LUTZ and Kenneth A. ARENDT*, Department of Biology, Boston University.

Petechiae are found in the cheek pouch of the hamster after exposure to ionizing radiation (x-rays applied to total body or cheek pouch, beta-emitting Sr^{90} glass beads implanted in the pouch, implanted Co^{60} needles). Following irradiation, increased susceptibility to petechial formation was demonstrated by counting the number of petechiae produced by 200 mm Hg negative pressure from a suction cup applied to the cheek pouch. The mechanism of petechial formation was studied and recorded by motion pictures taken through the microscope (900 ×) during actual production in the transillumined hamster cheek pouch and frog retrolingual membrane. For ease of continuous observation and photography, mocassin venom dissolved in mammalian Ringer's solution was applied topically to produce the petechiae. The blood flow in the venules became sluggish within one minute. Erythrocytes were concentrated and looked swollen, resembling strings of beads. Smooth muscle of arterioles constricted segmentally and some vasomotion was initiated. Numerous petechiae developed within 2 to 5 minutes, located chiefly at venous junctions. Red cells "popped out" one by one in spurts from a specific point on the blood vessel but without rupture of the wall or permanent opening. Extravasation ceased but not from platelet plugs.

[1] Supported by a grant from the Atomic Energy Commission.

MP 10. A cinematographic record of microscopical observations of the small blood vessels of the spiral ligament and stria vascularis of living guinea pigs.[1] Samuel R. GARGANO*, Francis L. WEILLE* and John W. IRWIN*, Medical Service of the Massachusetts General Hospital and the Winthrop Foundation of the Massachusetts Eye and Ear Infirmary. (Introduced by Edward H. Bloch)

The first part of the film will show a method to expose the spiral ligament and stria vascularis in living, anesthetized guinea pigs. Knisely's fused quartz-rod technic of transilluminating living tissue made possible the microscopic study of the blood vessels in this area.

One to four branching arterioles were found in the spiral ligament. Some of the branches fed a capillary network of the spiral ligament, whereas others ran deeper to supply blood to the capillaries of the stria vascularis. Still other branches were seen to pass directly into the venous system without an intervening capillary network. The two capillary networks were drained by collecting venules which in turn emptied into venules perpendicular to them. The film shows the flow of blood through all these vessels as well as a number of observations concerning the behavior of these vessels.

[1] Supported by the William S. Eaton Fund.

MP 2. Practical anatomy of inferior alveolar nerve block. Niels B. JORGENSEN* and John E. HUGHES*, Department of Anesthesiology, School of Dentistry, and Department of Anatomy, School of Medicine, College of Medical Evangelists. (Introduced by Otto F. Kampmeier)

A 15 minute excerpt from the film *Local Anesthesia in Dentistry, Part I, Mandibular Considerations.* Edward N. Hamilton, the producer, received the 1953 Honors Award for this film at the annual meeting of the Biologic Photographic Association, Inc., at the Los Angeles Statler Hotel.

The complete film presents demonstrations of accepted nerve blocks and infiltrations. Each demonstration is first shown on a skull for complete orientation with important landmarks. It is then further demonstrated on a dissection to show anatomical relationships of vessels and muscles. Finally the technique is performed on a patient. An important variation in the location of the lingular notch in Type III malocclusion is shown.

MP 3. Functional nerve regeneration between parabiotic rats. George E. METZ* and John C. FINERTY, Department of Anatomy, University of Texas, Medical Branch.

This is a 16 mm, Kodachrome, sound film illustrating the surgical techniques involved and the results of nerve anastomosis between one rat and its partner in parabiosis. The first sequence demonstrates the surgical procedure for parabiotic union according to the method of Bunster and Meyer (Anat. Rec., *57:* 339, 1933). The second sequence illustrates the method of nerve anastomosis which was carried out as originally described by Morpurgo (J. Physiol., *58:* 98, 1923). The sciatic nerves of adjacent legs of both animals were exposed, one being cut distally, in the region of the popliteal space. The other nerve was cut proximally, at the sciatic foramen. The nerves were then united by means of stainless steel wire, so as to have a continuous pathway from the spinal cord of one animal to the leg of the other. The third sequence describes the testing of the nerve. After a period of 3 months, painful stimulation applied to the innervated foot resulted in contraction of gluteal muscles of the other rat in an attempted withdrawal reflex of the denervated leg indicating the presence of sensory fibers in the regenerated nerve. Stimulation through the skin and on the exposed nerve proximal to the anastomosis with faradic current resulted in movement of the leg and foot of the other rat. Regeneration of the sciatic nerve between parabiotic rats confirms the early work of Morpurgo, and recent studies by Matzke and Kamrin (Science, *118:* 623, 1953).

MP 7. A cinematographic record on human peripheral blood leucocytes "in vitro" bearing on the question: Do leucocytes have a typical (glandular) secretory capacity? [1] Kenneth M. RICHTER, Department of Anatomy, University of Oklahoma School of Medicine.

Leucocytes are known to produce, both in health and disease, "de novo" neutral-red staining cytoplasmic droplets. Comparative and experimental cytologic studies (Ritcher, '40, '42) show them to arise topographically in association with the golgi complex. They have been considered generally as toxic or cyto-degenerative manifestations. Studies in this laboratory have revealed that in "in vitro"

preparations of whole blood neutrophiles, eosinophiles and monocytes within a matter of minutes begin a cyclic elaboration and extrusion of these droplets into the plasma medium where they retain their identities for undetermined lengths of time. The entire process resembles very closely the secretory cycle in merocrine gland cells.

Subjectively, the present film chiefly concerns (1) the extrusion phase of this cycle as it occurs in the neutrophiles and eosinophiles and (2) the simple physical characteristics and intra-cellular relationships of the droplets. The extrusion phase occupies approximately one second of time. The data recorded here, it is believed, comprises good cytologic evidence supporting the idea of a possible leucocytic secretory function originally suggested by Ranvier (Renaut, 1889).

The observations presented in this film were recorded at 8 frames per second through the dark-medium phase contrast microscope with high dry and oil immersion objectives.

[1] Supported by Grants-in-Aid from the American Cancer Society and the Helen Hay Whitney Foundation.

MP 6. Phase contrast cinematography of cells in vitro subjected to perfusion with "protective agents" and to freezing.[1] A. Cecil TAYLOR and Robert GERSTNER*, Department of Anatomy, Dental College, New York University.

Time lapse motion pictures offer a convenient means for studying the injurious effects of freezing upon cells in tissue culture and of the action of glycerol and ethylene glycol solutions when used as protective agents against such injury. Cultures of fetal rat skin were placed in a specially designed perfusion and freezing chamber and photographed with phase contrast illumination. The pictures show the changes taking place in individual cells as a result of freezing alone in comparison to those which accompany freezing after the protective perfusions. Picture sequences of the effects of perfusions alone show the response of cells to different durations of exposure to glycerol and ethylene glycol. Prolonged direct exposure, and especially the removal of the perfusate by direct washing with saline are shown to induce irreversible lethal changes in the cells. These changes become much milder and are reversible when the exposure time is reduced or when perfusion and washing are done through an interposed dializing membrane.

[1] Supported by a grant from the American Cancer Society on recommendation of the National Research Council, Committee on Growth to the Department of Anatomy, Dental College, and by a grant from the Atomic Energy Commission to the Plastic Surgery Unit, Department of Surgery, College of Medicine, New York University.

MP 9. Blood sludging resulting from thromboplastin injection in the dog.[1] John T. WILLSON* and Theodore S. ELIOT, Department of Anatomy, University of Colorado.

This film shows the development of intravascular agglutination in dogs receiving intravenous injections of thromboplastin. It is possible to adjust the rate of injection so that a state of afibrinogenemia is reached. When this point is passed, further thromboplastin injection does not observably alter the degree of sludging.

[1] Supported by a grant from the Office of Naval Research.

TISSUE CULTURE ASSOCIATION ABSTRACTS

TC 23. Tissue culture in vivo, using the transparent chamber technique in mice. Glen H. ALGIRE and James M. WEAVER*, National Cancer Institute, Bethesda.[1]

A modification of the transparent chamber technique has been devised in which a fragment of tissue may be implanted as a graft and yet be kept completely separated from cells and blood supply of the host by means of a membrane made of cellulose derivatives. Membranes of graded porosity, approximately 30 μ in thickness have been utilized in a system in which the implanted cells are maintained by diffusion of essential metabolites.

Microscopic observation of cellular activities may be observed in this system when the pores of a small area of the filter are caused to collapse through the application of a small drop of a solvent, amyl acetate, thus producing a transparent area in an otherwise relatively opaque material.

In results so far obtained, intracytoplasmic movement, rates of migration and time sequence of events during mitosis have been found, in general, to be similar to cellular behavior under conditions of *in vitro* tissue culture.

These methods have applicability to many problems in which one seeks to make comparisons between cellular activities *in vitro* and *in vivo*.

[1] National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

TC 15. Mitotic derangements in tissue culture caused by inhibitors of sarcoma growth in the mouse.[1] John J. BIESELE*, Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York. (Introduced by Thurlo B. Thomas)

Mitoses were studied in cultures of mouse sarcoma 180 and embryonic mouse skin fixed one day after dosing with agents known to be inhibitors of sarcoma 180 in the mouse, as well as with some ineffective analogs. Striking accumulations of blocked metaphases were noted in cultures treated with aminopterin or with its analogs that contained threonine or alanine in place of glutamic acid. Metaphase accumulation was not noted with 4-aminopteroyltryptophan, an agent ineffective against sarcoma 180 in the mouse. Chromosomal breaks and anaphase bridges were prominent effects of successful inhibitors containing ethylenimine groups, including both triazines and phophoramides. Similar chromosomal effects were noted with 2, 4, 6-tris-(2-vinylaziridino)-S-triazine, in which the ethylenimine groups are substituted, despite the ineffectiveness of this agent *in vivo*. Replacement of the two ethylenimine groups in the inhibitory diethylamide diethylenimide of phosphoric acid with ethyl groups in ester linkage to form the non-inhibitory diethyl N, N-diethylamidophosphate also eliminated the predilection to cause chromosomal damage in tissue culture.

It is concluded that no overall uniformity in mitotic derangements in tissue culture marked this set of successful inhibitors of the growth of sarcoma 180 in the mouse.

[1] Supported by grants from the American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research, and by grant C-678 (C5) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

TC 1. Some investigations on the relations between growth and carbohydrate metabolism.[1] Sjoerd L. BONTING[2] and Marion JONES, Departments of Physiology and Bacteriology, College of Medicine, State University of Iowa, Iowa City.

Consumption of glucose and the production of lactic acid and pyruvic acid have been determined in cultures of chick lung and intestine under different experimental conditions. Amounts of 20–30 mg of finely minced tissue were cultivated in embryo extract in roller tubes for 4–10 days. Growth was determined as increase in protein. Special ultramicrochemical methods for the determination of glucose, lactic acid, and pyruvic acid were developed and have been employed in these studies. Beause of high acid production under anaerobic conditions, a new salt solution with high buffer capacity was developed and used.

Lung and intestine cultivated under nitrogen showed approximately the same protein production as under air. Glucose consumption was increased somewhat, while the glucose consumed could be essentially accounted for as lactic acid produced. Cultures in air showed only small or no lactic acid production. Cultures in oxygen showed a smaller increase in protein with the lower glucose consumption and higher lactic acid production.

Cyanide added to the system in concentrations that blocked respiration permitted growth to continue, with the glucose consumed being accounted for almost completely by lactic acid produced. Under none of the experimental conditions did appreciable accumulation of pyruvic acid occur.

These experiments show that while oxidative glucose breakdown is the main source of energy under aerobic conditions, the non-oxidative pathway can supply sufficient energy for growth in embryonic tissues under anaerobic conditions.

[1] Supported in part by grants from the Iowa Division, American Cancer Society and the Central Scientific Fund of the College of Medicine of the State University of Iowa.
[2] Postdoctoral Research Fellow of U. S. Public Health Service (National Cancer Institute).

TC 17. Megaloblasts of chicken embryo, observed in the living state. E. BORGHESE* E. G. RONDANELLI*,° and E. STROSSELLI*,° Departments of Anatomy and Clinical Medicine, University of Pavia, Pavia. (Introduced by Esther Carpenter)

Circulating blood of chicken embryos at 60–70 hours of incubation containing megaloblasts only, was observed as a thin layer between slide and coverslip, or mixed with a drop of chicken plasma and a drop of chicken embryo extract, in order to form a clot. Phase contrast microscopy and cinematography were employed.

The megaloblasts, usually big, round cells, showed a round nucleus, with a well defined nuclear membrane, and one or two nucleoli. In the cytoplasm chondriosomes were recognizable, as granules or small rods, not uniformly scattered but more or less concentrated around the nucleus. Sometimes these chondriosomes occurred all around the nucleus or sometimes in a sort of Golgi zone, continuously moving and changing their shape.

Not all megaloblasts were regularly round: some of them protruded and retracted lobes, which correspond to those already known in the literature as pseudopodia. Motion pictures have shown that such cytoplasmic protrusions can appear

under normal conditions but never behave as pseudopodia in the usual sense, viz. as locomotor organs: the cells under their action do not change place in the field.

At the surface of such megaloblasts, especially when they are in a plasma clot, one can observe the protrusion of filaments similar to those described in human blood by several authors under the name of "haematexodies." Prolonged observation and accelerated microcinematography have shown that such filaments move and change their shape and length continuously, becoming short and long and transforming in granular protrusions around the cells and vice versa.

TC 4. Influence of various culture media on the growth of Earle's L-strain and chick heart fibroblasts in vitro.[1] Relda CAILLEAU* and Paul L. KIRK*, Department of Biochemistry, University of California, Berkeley.

Prolonged cultivation of Earle's L-strain of mouse connective tissue has been obtained in the absence of embryo extract. Media containing Morgan, Morton and Parker's synthetic mixture 199, with the addition of horse serum or ascitic (or plural) fluid of malignant origin seem capable of maintaining the L-strain indefinitely. Successful cultures have been carried out over a period of more than six months, with frequent subcultures producing an increase in cell population of 5 to 10 fold over the initial inoculum. Cell appearance varies in the two media. The L-strain cells appear normal in the medium containing horse serum but appear small, round and clear in the presence of ascitic fluid (or pleural fluid). In the latter media the cells lose much of their ability to adhere to the glass and usually float freely in the medium in the form of spheres.

Fresh explants of chick hearts grown in a plasma clot are incapable of producing normal fibroblasts in the above media in the total absence of chick embryo extract. Growth is minimal and abnormal, and subcultures are unsuccessful.

[1] Supported by a grant from the National Cancer Institute, National Institutes of Health, Public Health Service.

TC 16. The physiological activity of chick embryo thyroids grown in vitro as indicated by uptake of radioactive iodine. Esther CARPENTER, J. BEATTIE° and R. D. CHAMBERS*,° Strangeways Research Laboratory, Cambridge.

Thyroid glands from chick embryos of 8 days of incubation were grown in pairs on the surface of the clot by the coverslip method. On the 7th day in vitro 0.01 ml of Tyrode's solution containing 0.01 microcurie of ^{131}I was spread over the surface of the clot and the culture resealed. In each of three experiments, after 24 hours of exposure to the isotope, 8 pairs of glands were transferred to a weighing bottle and weighed. Fragments of each clot were pooled also and weighed; the volume was made up to 0.2 ml with 10% NaOH solution, and the material dissolved by heat on a water bath. As determined on dried aliquot parts of each solution, the ratio of radioactivity of thyroid cultures to that of the clot was 296, 102, and 275 to 1 in the three experiments. Embryos of the second series were underdeveloped compared with those of the other two. In the third series, pieces of esophagus grown on the same clots as the thyroids for the last 4 days gave counts even lower than those of the culture medium.

Eggs incubated 7 and 15 days were injected with 0.5 ml Tyrode's solution containing 10 microcuries ^{131}I. Thyroid glands and samples of amniotic fluid were removed 24 hours later and their radioactivity determined. Thyroids from 8 day embryos concentrated ^{131}I to only a slight degree, but those from 16 day embryos gave concentration ratios of the same order of magnitude as thyroids from 8 day embryos grown 8 days in vitro.

In all series, glands exposed and not exposed to ^{131}I were fixed for autoradiographs and histological study. Autoradiographs showed no blackening of the film by thyroids from 8 day embryos: intense blackening by those from 16 day embryos; and distinct but less intense blackening by those grown 8 days in vitro. Gaillard ('53) has obtained positive autoradiographs of thyroids from 8 day embryos grown 2 days in a medium containing radioactive iodine.

TC 21. Action of Coxsackie virus on contraction of chick embryo muscle cultivated "in vitro." Eugenia S. de LUSTIG*, Sección Virus, Instituto Malbrán, Buenos Aires. (Introduced by Katherine Sanford)

Coxsackie virus isolated during a local epidemic in 1953, when added to "in vitro" cultures of striated muscle of chick embryos from 8 to 15 days' incubation age, produced paralysis of the automatic contractions together with vacuolation and degeneration of the myoblasts. Histologically one observed edema of the sarcoplasm, loss of striation, and displacement of the nuclei toward the center of the fiber.

TC 19. Wound healing in tissues cultivated in different sera. Eugenia S. de LUSTIG* and Fabio SACERDOTE*, Instituto de Medicina Experimental and Centro de Investigaciones Tisiológicas, Buenos Aires. (Introduced by Wilton R. Earle)

The alkaline phosphatase activity (cytochemical) of the cells growing into a regenerating wound of a culture of chick embryo fibroblasts is higher than in the cells previously migrated in the growth area. As in the whole organism during regeneration and as in embryos during differentiation, there seems to exist also in a colony of cultivated cells, a relationship between alkaline phosphatase and wound healing.

The healing of a wound produced in a culture is really a regeneration within a regenerating system, only that in the culture it takes place at a higher rate. In the first 24 hours, fibers (silver technique) and mitoses are already observed, simultaneously with strongly positive phosphatase reaction. We confirm thus in our wounded cultures, the results of Chèvremont and Firket, who state that the intensity of the phosphatase reaction is proportional to the mitotic activity. We confirm as well the relationship between fibrous protein formation and alkaline phosphatase activity of fibroblasts (Danielli; S. de Lustig and Sacerdote).

The newly formed cells of a wound respond more readily than the previously migrated cells to the action of different normal and pathological human sera. The higher mitotic index, stronger phosphatase reaction and shorter healing time point to a high sensitivity of these wound cells to small changes in the nutritional environment.

TC 20. Action of nitrogen mustard on normal and neoplastic cells cultivated "in vitro." Eugenia S. de LUSTIG*, Angélica TEYSSIE*, Arnoldo MILNER* and Fabio SACERDOTE*, Instituto de Medicina Experimental and Centro de Investigaciones Tisiológicas, Buenos Aires. (Introduced by Duncan C. Hetherington)

The cells of cultures "in vitro" are sensitive to nitrogen mustard at concentrations from 1×10^{-4} to 1×10^{-5} gm/cc. The nucleus, both mitotic and resting, is the cell constituent most markedly injured by nitrogen mustard. The most sensitive phase of mitosis is the prophase. The destruction of the nuclear membrane is demonstrated by the presence of alkaline-phosphatase-positive granules which have diffused from the nucleus to the cytoplasm. Higher concentrations of nitrogen mustard inhibit normal growth and destroy the cell membrane. Explants seriously injured by strong concentrations lose completely their power of migration, even after washing. Normal embryo fibroblasts are more sensitive than normal embryo myoblasts. The growth of the free ends of the muscle buds proceeds normally even in the presence of degenerated fibroblasts. Cells of methylcholanthrene sarcoma suffer to a less generalized degree than do normal fibroblasts. The nuclear membrane, which disappears completely in normal chick fibroblasts, very seldom does so in the sarcomatous fibroblasts. Adenocarcinoma cells, which grow as an epithelial membrane, suffer to a more generalized degree than do sarcoma cells. We are studying cytochemically the action of British Anti-Lewisite on normal and on tumor cells previously treated with nitrogen mustard.

TC 22. Action of Coxsackie virus on an experimental mouse tumor cultivated "in vitro." Eugenia S. deLUSTIG*, Sección Virus, Instituto Malbrán, Buenos Aires. (Introduced by M. Chèvremont)

A Coxsackie virus, isolated in Argentina in 1953, is able to infect mouse sarcoma fibroblasts cultivated "in vitro." The infected malignant cells show rosette-shaped basophilic inclusions in the nucleus and their filamentous mitochondria are extremely thin.

Adult mice generally refractory to this viral infection become susceptible after being inoculated with a culture of sarcoma cells infected with Coxsackie virus.

TC 9. A further comparison of the activity of possible antineoplastic compounds "in vitro" and "in vivo." P. A. EICHORN*, K. V. HUFFMAN*, J. J. OLESON*, S. L. HALLIDAY* and J. H. WILLIAMS*, Lederle Laboratories Division, American Cyanamid Company, Pearl River.

The present study is an extension of a program designed to elucidate the possible validity associated with the use of cultured animal cells as a means of screening substances for antineoplastic activity. A previous report (Ann. N. Y. Acad. Sci., Jan. 1953) indicated that results from an *in vivo* and *in vitro* screening of eight chemical compounds correlated very closely. The encouraging results from this study initiated a further investigation into this comparative relationship.

Additional unrelated chemical compounds, varying in solubility and, in most instances, known only by a code number, were tested for their toxicity, selectivity of action against tumor cells in comparison with normal cells, and their selectivity of action against specific tumors. These substances were independently tested *in vivo* and *in vitro* on two types of animal tumors. The conclusions of activity identified by the two methods were once again strikingly similar.

Several factors relating to *in vitro* evaluations and concerned with the solubility of the compounds as well as the significance of data indicating selectivity of action against tumor cells will be discussed.

TC 6. Passive transfer of tuberculin allergy to normal leucocytes by plasma from tuberculous humans. C. B. FAVOUR* and E. F. O'NEILL, Jr.*, Department of Medicine, Harvard Medical School, Boston, and Veterans Administration Hospital, Rutland.

Washed peripheral blood cell suspensions from normal and tuberculous subjects freed of autologous plasma and of platelets were suspended in plasmas from normal and tuberculous subjects. Parallel suspensions containing varying concentrations of cells and suspending fluid replicate microhematocrits were formed in capillary tubes and incubated overnight. Linear migration against gravity of buffy coat leucocytes was recorded for statistical analysis. The cultures from some patients grown in autologous plasma showed significant specific inhibition by tuberculin of cell migration, compared to normal cells in their own plasma. Washed leucocytes from these selected "positive" subjects in the presence of tuberculin and normal plasma showed an occasional inhibition of migration. Washed normal leucocytes in "positive" plasma and tuberculin were specifically inhibited. Appropriate cell, plasma, and antigen controls showed normal cell migration. These findings indicate that tuberculin toxicity for leucocytes from tuberculous humans is mediated by a plasma factor. Carefully washed leucocytes from patients retain little ability to respond to tuberculin *in vitro*.

TC 13. The response of ovaries in vitro to FSH.[1] Mary J. GUTHRIE, Detroit Institute of Cancer Research, Detroit. (Introduced by Wm. L. Simpson)

Ovaries of recently born mice of the inbred Marsh strain can be grown as organs on heterologous plasma clots, using the watch-glass method. A response to minute amounts of several different compounds can be detected by comparing the number of dividing cells in the two members of a pair of ovaries grown under different conditions.

In order to determine if the cells of the mouse ovary, particularly the very young ovary, were directly responsive to the hypophyseal follicle stimulating hormone, the members of pairs of ovaries of sibling mice were explanted to pairs of clots, one of which in each series was fortified with Armour's Pituitary Gonadotropin (FSH). Litters from a few hours post-partem up to 48 hrs. after birth were used. The FSH level in the clots ranged from 0.05 to 0.50 Armour units. After incubation for 3 to 7 days, the clot was flooded with a dilute solution of colchicine for a period of 4 to 6 hours. The arrested metaphase plates were counted in serial sections.

At concentrations of 0.15 to 0.30 Armour units the enhancement of mitotic activity in series of ovaries from sibling mice ranged from 30 to 100%. In the experiments described, the explanted and immature mouse ovary is directly stimulated by FSH in appropriate concentrations.

[1] Aided by grants from the American Cancer Society and USPHS.

TC 3. Effects in tissue culture of a nucleoprotein fraction isolated from various embryonic organs. Roman KUTSKY* and Relda CAILLEAU*, Donner Laboratory of Medical Physics and the Biochemistry and Virus Laboratory, University of California, Berkeley.

A method has been developed by Kutsky (P.S.E.B.M., *83*, 390, 1953) for isolating an active nucleoprotein fraction from whole chick embryo extract. To determine if this fraction is localized in certain regions of the 12 day chick embryo, various organs of 12 day chick embryos were dissected out and collected in separate batches. Using the above method, a corresponding nucleoprotein fraction was then isolated from each organ extract. These fractions were tested for outgrowth promoting activity in cultures of chick heart fibroblasts in Carrel flasks and in roller tubes. On the basis of ultraviolet absorption equal concentrations of the tissue nucleoprotein fraction were tested in culture. This concentration level was sufficiently high to elicit a favorable outgrowth response in terms of surface area and density of cells when whole embyro nucleoprotein fraction was tested. Of the organs tested (heart, gut, liver, brain, spinal cord, etc.) the spinal cord and brain showed an enhanced outgrowth while the heart was inert or inhibitory in its effect. In general, in cultures of the active organ nucleoprotein fractions, the cell morphology closely resembled that of the cultures supplemented with the whole embryo nucleoprotein fraction. These results indicate that the whole embryo nucleoprotein fraction consists of several nucleoprotein fractions with differing biological activity. Parallel experiments are in progress testing these fractions on mouse cells in tissue culture.

TC 11. In vitro experiments on a nerve growth-promoting agent in mouse sarcoma 37 and 180. Rita LEVI-MONTALCINI, Stanley COHEN* and Viktor HAMBURGER, Department of Zoology, Washington University, St. Louis.

When mouse sarcoma 37 and 180 are transplanted onto the allantoic membrane of four-day chick embryos the sympathetic ganglia of the chick are highly hyperplastic and supernumerary ganglionic masses are formed. Nerves emerging from these ganglia invade the viscera and enter the blood vessels of the embryo. It was concluded that the effect is produced by a diffusible agent (Levi-Montalcini, Ann. N. Y. Acad. Sci., 330–343, 1952).

Tissue culture was used for the analysis of this agent. Sensory and sympathetic ganglia of chick embryos (6–13 days of incubation) were combined with small pieces of sarcoma 37 or 180. After 24 hours the ganglia show a conspicuous radial outgrowth of nerve fibers whose density is maximal on the side toward the tumor. Control cultures in which the same ganglia were combined with nor-

mal embryonic chick tissue show only few nerve fibers but a large number of migrating fibroblasts.

The nerve growth-promoting agent was now found to be present also in cell-free homogenates of these tumors. Sympathetic and sensory ganglia growing in a standard medium to which tumor extract was added produce within 12 hours the same type of fiber outgrowth which was observed in the previous experiments with tumor pieces. Tests with fractions of the homogenates obtained by centrifugation in a medium of .25 M. sucrose at pH 7.4 showed a maximal activity in the microsome fraction of the tumor cells.

TC 10. Electron microscope study of nerve fibers grown "in vitro." Herta MEYER*, Instituto de Biofisica da Universidade do Brasil, Rio de Janeiro. (Introduced by Mary S. Parshley)

Nerve fibers of spinal ganglia of chick embryos, grown in vitro, have been examined with the electron microscope. A fine longitudinal striation has been observed in the cytoplasm which reaches up to the growing end of the fiber. The fibrils which are responsible for the striation show parallel arrangement and have a diameter of 250–400 A. They show a regular periodicity where the fiber is thick and compressed. This produces a more beaded appearance where the fiber spreads to form its terminal expansion.

The terminal expansions of the growing fibers are more complex than can be seen with the light microscope. Besides amoeboid membranes, numerous fine prolongations are present. Often inclusion bodies, similar to mitochondria, can be found in them and also empty spherical spaces recalling vacuoles.

TC 7. Plasmodium gallinaceum in tissue cultures: Observations after one year of cultivation. M de Oliveira MUSACCHIO* and Herta MEYER*, Instituto de Biofisica da Universidade do Brasil, Rio de Janeiro.

The exoerythrocytic form of P. gallinaceum has been cultivated in tissue cultures for one year. The cultures showed the same high grade infection as has been observed with Toxoplasma and with Trypanosoma Cruzi so that new observations upon the living parasite and the cell-parasite relationship were possible.

It has not been possible to obtain "in vitro," the passage from the exo- into the endoerythrocytic form.

The parasites can survive in tissue cultures which are kept at room temperature for more than 20 days. The cycle under these conditions is delayed.

TC 5. The gradient culture.[1] Edwin E. OSGOOD* and M. Larsen KRIPPAEHNE*, Division of Experimental Medicine, University of Oregon Medical School, Portland.

Since it had been observed in some culture work that the number of cells per unit volume of culture medium, the depth of layer, and the oxygen tension were all critical factors, a method was devised which gives a continuous gradient in all these factors so that at some level conditions are almost certain to be right. The method consists of inserting a sterile glass slide at a 45° angle in a sterile 1-pint

French square bottle containing 125–170 ml of suspension type culture. Slides may be removed and the cells examined in situ at any desired interval. By this method cultures of normal and of leukemic hemic cells have been far superior to cultures obtained by any previous method, and it has been possible to culture cells from the blood of patients with various types of acute leukemia, something which had been impossible with other method.

Different cell types require different conditions. No cells so far tested have grown well at depths of less than 2 cm. Optimal depths for cells of the granulocytic and monocytic series seem to be about 3–3½ cm and for cells of the lymphocytic and erythrocytic series 4–5 cm. The growth patterns on these slides resemble those in the hematopoietic organs and will be illustrated with lantern slides.

[1] Supported in part by grants from the U. S. Atomic Energy Commission and the National Cancer Institute of the U. S. Public Health Service.

TC 14. The development of tubular structures in cultures of epithelium from the cortex of adult dog kindey.[1] Mary Stearns PARSHLEY, Department of Surgery, Columbia University.

Tissue cultures of epithelial cells from adult dog kidney cortex have been maintained in a fluid medium in Carrel flasks without transfer for over two months. The cultures were planted in a thin clot of chicken plasma with a supernatant fluid medium of 33% dog serum and 10% dog spleen extract at pH 7.4. The plasma, which was liquefied completely within 24 hours, was not replaced. The explants remained suspended in the fluid medium which was renewed at weekly intervals.

Two kinds of growth occurred. Knobs of epithelial cells and thick-walled tubular structures 7–8 mm in length grew out from the surface of the floating explants, which appeared also to shed cells into the medium. These cells formed centers for the development on the glass bottom of the flask of extensive sheets of epithelium, which measured up to 12 × 20 mm at 8 weeks. Mitosis was observed rarely. The many binucleate cells present may indicate amitotic division as well. Extensive crude tubular structures which formed in the sheets were observed to contain a red fluid presumably phenol red taken up from the medium and secreted into the lumen.

It is believed that this is the first report of the maintenance in culture of adult mammalian kidney epithelium over an extended time with the development from sheets of epithelial cells of apparently functioning tubules.

[1] Supported by a grant from the New York Heart Association.

TC 2. The effect of insulin on nucleic acid synthesis and carbohydrate metabolism in tissue culture. John PAUL*[1] and Ian LESLIE*, Department of Biochemistry, Glasgow University. (Introduced by Margaret R. Murray)

Uniform explants of chick-embryo heart were cut mechanically and grown in special roller-tubes, incorporating a microtest-tube. The medium contained varying concentrations of insulin and was changed daily. At the end of seven days the tissues were scraped into the test-tube portion of the roller-tube and analysed chemically for protein, phospholipid (LP), ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The collected media were also analysed, for glucose, pyruvic acid and α-ketoglutaric acid.

At high concentrations (10^{-1} to 3 units/ml medium) insulin greatly stimulated the formation of protein, LP, RNA and DNA and elevated the ratio RNA/DNA. There was increased utilisation of glucose. This was accompanied by a profound fall in the concentration of pyruvic acid in the medium while the level of α-ketoglutaric acid was unaltered. These trends became less marked as the insulin concentration was lowered and at 10^{-4} units/ml medium they were no longer significant.

It is concluded that insulin directly stimulates the growth of chick-heart fibroblasts in tissue culture and that it causes increased energy production and synthesis through its control over pyruvate metabolism.

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TC 18. Measurement of cell transformations within transplants of tissue cultures.[1] Hans H. PFEIFFER*, Laboratory for Polarization Microscopy, Bremen. (Introduced by Giuseppe LEVI)

Results of recent experiments prove the dependence of cell shape upon the physical structure of the transplant and the tensile trajectories within it and express this *dependence in quantitative form.* Standard tissue cultures of the upper pharyngeal ganglia from the third thoracic segment of larvae of *Drosophila melanogaster* MEIG. or of parts of the infra-esophageal compound ganglion were grown in Fischer and Gottschewski's medium. As in former experiments (Pfeiffer, Naturwiss., *31:* 47, 1943), M. Vanyo's technique (Arch. exper. Zellforsch., *24:* 105, 1940) was changed using extensible leaflets of cellophane sterilized in Lugol solution, decolorized in 5% sodium thiosulphate and affixed at the culture chamber parallel to or vertical to their direction of tension. After a certain experimental time, the leaflets were turned for 90 degrees. By this means it is possible to produce a quantitative gradation of the tension and a perfect reorientation within the transplant. These variations were established by measuring the *birefringence* with a variable-azimuth compensator of fixed retardation, viz. a rotating λ/30 mica plate, and a rotating half-shadow wedge of Macé de Lepinay, and the results were substantiated by measurements of *dichroism* (technique of Pfeiffer, Expt. Cell. Res., *5:* in press 1953) after Weber's $AgNO_3$ impregnation. Beyond the demonstration of tensile gradations and the morphologic tissue transformation it is possible to derive the nature and direction of the observed quantitative variability from consideration of a texture of the transplant, particularly of its tensile trajectories.

[1] Supported by a grant from the Foundation of Kolloid-Ges.

TC 8. Studies on the individual and joint effects of histamine-hydrochloride and chlor-trimeton on cultures of embryonic chick heart.[1] Kenneth M. RICHTER, Department of Anatomy, University of Oklahoma School of Medicine, Oklahoma City.

Cultures of 10–12 day embryonic chick hearts were used to determine the individual and joint effects of histamine-hydrochloride and chlor-trimeton at concentrations of .1 and .01 mgm/cc of nutrient medium during the course of 48 hours of cultivation.

At concentrations of .01 mgm/cc, histamine-hydrochloride and chlor-trimeton singly and in combination did not significantly alter from normal (1) the pulsatile contraction of heart fragments or (2) the general growth and migration of cells from the explants. Phase contrast studies likewise revealed that these drugs at this concentration when used singly or in combination did not effect discernible changes from normal in the appearance of the nuclei, Golgi complex or mitochondria.

At concentrations of .1 mgm/cc of nutrient medium, histamine-hydrochloride and chlor-trimeton individually and jointly significantly inhibited (1) the pulsatile contraction of heart fragments and (2) cell growth and migration. The inhibitory action of histamine-hydrochloride on cell migration and growth was only slightly lessened by concurrent exposure to chlor-trimeton.

Time-lapse cinematographic studies showed the principal cytomorphic changes effected by histamine-hydrochloride to be a vacuolation of the cells with increased cell surface activity, followed by an eventual rounding-up and loss of surface activity. A short film of the effect of histamine-hydrochloride on heart fibroblasts as recorded with the dark-medium phase contrast miscroscope will be shown.

[1] Supported by a Grant-in-Aid from the Helen Hay Whitney Foundation.

TC 12. Tissue interactions. Joseph J. WORZNIAK*, Wyandotte, Mich. (Introduced by Kenneth L. Duke)

Various procedures were done to rule out non-physiologic reasons for the tissue interactions reported at the last meeting. The serum content of the medium as well as explant size and proximity were varied. Heated and plain media from tubes containing one tissue type were transferred to tubes containing the "test" tissues. Individual organ extracts were used with the medium in the "test" tissue tubes.

The suppressive action that bowel, lung, and liver explants exert on fibroblasts, but not epithelium, can be reversed by patching, by chicken serum, and less effectively by horse serum. Proximity of larger heart and muscle explants or addition of some medium from a tube in which heart or muscle explants are growing in the absence of serum has the same effect.

With the increased fibroplasia produced by these methods changes in the epithelial growth occur. These changes range from suppression, to detachment of fragments, fading of cellular detail, vacuolization, etc. Serum, in the absence of fibroblasts, has no deleterious effect on epithelium. These events help explain the classical observation that fibroblasts "outgrow" epithelium.

Tissue interactions are masked by high serum concentrations and thick plasma clots. They are effected through heat labile substances originating in proliferating cells. The events cannot be duplicated by extracts of individual organs. They appear to represent physiologic interactions.

RUBRO-CEREBELLAR CONNECTIONS

AN EXPERIMENTAL STUDY IN THE CAT

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Anatomical Institute, University of Oslo, Norway

FOURTEEN FIGURES

INTRODUCTION

The existence of fibers passing from the dentate nucleus via the brachium conjunctivum to the red nucleus has been repeatedly demonstrated. As far as we have been able to ascertain, fibers coursing in an opposite direction, i.e. neurites of cells of the red nucleus passing to the cerebellum, have not been generally recognized, although some authors have advocated their presence (Forel, 1881; Gudden, 1882; Vejas, 1885; Mahaim, 1894; von Monakow, '09). When studying more systematically the sites of origin of cerebellar afferents from the brain stem in the cat, one of us (A.B.) noticed characteristic retrograde (axonal) changes in cells of the red nucleus following experimental lesions of the cerebellum. Since this finding was made consistently in several animals, the present study was undertaken in an attempt to elucidate this pathway more closely.

MATERIAL AND METHODS

Altogether 22 experimental animals were employed in this study. The brains of three normal cats served as control. Some of the experimental animals have been used also in other studies of afferent cerebellar connections (Brodal, '52, '53).

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The modified Gudden method worked out previously (Brodal, '40) was employed. The cats were operated on at the age of 6–18 days and killed 4–10 days later. Under nembutal anaesthesia and under sterile precautions various lobules and parts of the cerebellum were removed by means of knife and suction. The animals were killed by exsanguination under an overdose of nembutal or chloroform, the brain and brain stem immediately dissected free and immersed in 96% alcohol for fixation. Following adequate fixation the cerebellum and the attached brain stem were embedded in paraffin and sectioned serially at 15 μ in the transversal plane. Every 5th section was mounted and stained with Thionine. When it proved desirable, intervening sections were mounted and stained later.

The sections passing through the red nucleus were searched for nerve cells presenting retrograde cellular changes. In these young animals the cells, the neurites of which have been damaged, display clearcut alterations in the course of a few days and will disintegrate completely during the following two to three days. Since a moderate cell loss will be difficult to detect, it is essential to kill the experimental animals when the cellular changes are at their maximum. This was found to be about 5 days following the cerebellar ablation. However, on account of the considerable individual variations in the rates of the changes (see Brodal, '39, for particulars), it may happen that in certain cases the cellular changes are too slight to be identified, while in other animals having survived for a similar period most of the altered cells have already disappeared. The use of a larger number of animals enabled us to overcome the difficulties due to these individual variations.

OBSERVATIONS

Before presenting the experimental evidence brief comment on the normal topography and cellular architecture of the red nucleus in the cat is appropriate, as is also a short description of the cellular changes in the experimental animals.

I. The normal red nucleus in the cat

The red nucleus of mammals is generally considered as composed of a large-celled caudal part and a small-celled rostral part. Descriptions of the red nucleus in the cat have been given by Hatschek ('07), von Monakow ('09), Winkler and Potter ('14) and by Davenport and Ranson ('30). Von Monakow distinguishes altogether 5 subdivisions of the nucleus. Winkler and Potter recognize three, while Davenport and Ranson maintain that it is only possible to distinguish a caudal compact part and a rostral reticular part. It is clear from our material that there are considerable individual variations in the architecture of the red nucleus in the cat, making attempts at a finer subdivision arbitrary. In view, furthermore, of the gradual changes in its picture from level to level, there seems to be little justification for distinguishing separate parts. On the whole our conception of the red nucleus is in agreement with that advocated by Davenport and Ranson.

Figure 1 shows 5 camera lucida drawings of transverse sections through the mesencephalon of a 15 day old cat. The drawings are taken with equal intervals. The distribution of cells of different sizes at these 5 levels is shown to the right. Camera lucida drawings have been checked under the microscope, and all cells observed in the particular sections are indicated, their relative sizes being shown approximately by the dimensions of the dots. As described by several previous authors, among others by Cajal ('09), three types of cells, large, medium-sized and small ones may be distinguished in the red nucleus. Specimens of the different types are seen to the left in the drawing of figure 7, which makes a description superfluous.

When traced from caudal to rostral levels the red nucleus begins with a rather compact caudal end, made up almost exclusively of cells of the largest type, with only a few medium-sized cells intermingled (fig. 8). From this compact, large-celled caudal pole there is a gradual transition in a

rostral direction. There is a decrease in the number of large cells, which are by and by substituted by medium-sized and these again by small ones. Even at rostral levels, however, scattered cells of the large type occur. Also the average diameter of the cells of each type appears to decrease steadily. Thus the scattered large cells at rostral levels are distinctly smaller than their fellows at caudal levels. Concomitant with these changes in cellular composition there is a progressive change from a compact to a more loose reticular structure of the nucleus, as will be seen from figure 1. The rostral pole of the nucleus fuses almost imperceptibly with the reticular formation surrounding it.

It will be seen from the following description that the distribution of altered cells following cerebellar lesions does not betray any particular topographic subdivision of the nucleus, nor does the termination of afferent fibers to the red nucleus, as far as can be judged from the available literature. There seems to be little reason, therefore, to distinguish in the cat more than two chief parts of the nucleus, fusing gradually

ABBREVIATIONS EMPLOYED IN ALL FIGURES

Br.c., brachium conjunctivum
Br.p., brachium pontis
C_1, lobulus C_1 of Bolk, pyramis
C_2, lobulus C_2 of Bolk
C.m., mammillary body
C.r., restiform body
Cr. I and Cr. II, crus primum and secundum of ansiform lobule of Bolk
C.s., superior colliculus
Flocc., flocculus
F.r., fasciculus retroflexus
L., left
L.ant., anterior lobe
L.a.p., anso-paramedian lobule
L.p.m., paramedian lobule
m., nucleus minimus
N. III, VI, VII and VIII, oculomotor, abducent, facial and vestibular nerve resp.
N.d., nucleus dentatus
N.f., nucleus fastigii
N.i., nucleus interpositus
N.i.p., nucleus interpeduncularis
N.n. III, nucleus of oculomotor nerve
N.n. V, sensory nucleus of trigeminal nerve
N.n. cochl., nuclei of cochlear nerve
N.n. vest., nuclei of vestibular nerve
Nod., nodulus
N.r., red nucleus
Ol.s., superior olive
Ped.c., cerebral peduncle
P.fl., paraflocculus
Po., pons
R., right
S.n., substantia nigra
S.r.m., reticular substance of mesencephalon
Uv., uvula

one into the other: a caudal, magno-cellular, rather compact part and a rostral, parvi-cellular, more reticular part. However, in section 3 of figure 1 a small lateral extension of the nucleus is seen, forming a fairly well circumscribed group of rather densely packed small cells. This group which is clearly to be distinguished in most animals appears to cor-

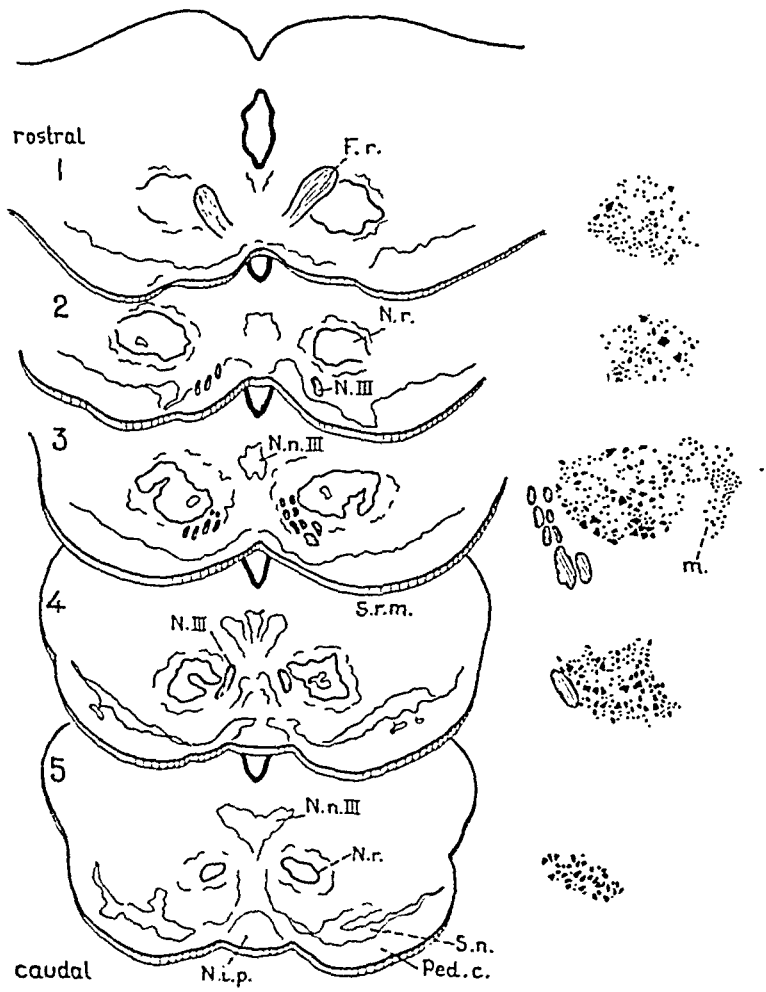


Fig. 1 Diagram (made by means of a projection apparatus) showing the normal topography and cyto-architecture of the red nucleus as seen in transverse Nissl-stained sections through the mesencephalon of a two week old cat. The drawings are taken with equal intervals. To the right are shown drawings at higher magnification of the left nucleus at the levels represented. The three types of cells present are indicated by dots of different sizes (drawings checked under the microscope).

respond to the nucleus minimus of von Monakow ('09). However, since our preparations do not reveal changes in this, we shall not consider it here.

II. Experimental results

(a) The retrograde cellular changes in the red nucleus following cerebellar lesions. The changes observed in the cells of the red nucleus following certain cerebellar lesions (vide infra) are of the same type as those seen in the same experimental animals in other nuclei sending their fibers to the cerebellum, and which have been described previously (for example in the external cuneate nucleus, Brodal, '41; the lateral reticular nucleus, Brodal, '43; the paramedian reticular nucleus, Brodal, '53; the pontine nuclei, Brodal and Jansen, '46). Representative specimens of altered cells are reproduced in figure 7, and in the photomicrographs of figures 9–12. The chief alteration consists of a tigrolysis, which appears to begin centrally but later affects the entire cytoplasm, leaving only a few Nissl granules along the periphery of the cells. The tigrolytic cytoplasm acquires a homogenous, milky appearance, staining a faint bluish hue in Nissl preparations. The nucleus is displaced to the periphery of the cell. In normal animals of the same age the nucleus may also be somewhat peripherally situated in some cells, but never extremely so, while in many of the altered cells the nucleus appears to be on the point of being extruded. Sometimes the nucleus changes its shape, becoming flattened or bean-shaped, and in more severely changed specimens the nucleolus may be diminished and the nuclear membrane indistinct. On account of the varying size of the cells, it is difficult to decide whether there is any change in volume, but indications are that there takes place a certain amount of swelling, with a rounding off of the cell contours.

The time course of the alterations shows considerable variations, but in most animals the changes are fully developed 4–5 days after the cerebellar lesion has been made. Then the

cells appear to disintegrate rather rapidly, but the later stages have not been studied.

The changes described are most easily observed in the large cells of the red nucleus, but they are also clearcut in the medium-sized ones (see figs. 7 and 10). Convincingly altered cells of the small type may also be seen, but frequently there may be some doubt whether a particular cell is pathological or not, since the small amount of cytoplasm makes it difficult to ascertain moderate degrees of tigrolysis and a possible displacement of the nucleus.

In the experiments to be described below, only cells presenting unequivocal changes of the type described have been taken into account. This probably means that some cells being slightly changed may have been left out of consideration. Even if the alterations registered will thus represent the minimum of changes and perhaps not the totality, this procedure was deemed preferable to running the risk of drawing conclusions on the basis of possibly equivocal cell changes.

(b) The rubro-cerebellar projection. Maximum of cellular changes in the red nucleus following cerebellar lesions is seen in animals in which the entire cerebellum has been removed. The following case is described as an example.

Cat O 131. Age at operation 10 days. Killed 8 days later (Figs. 2, 9 and 10)

Lesion. By removing part of the occipital bone above the foramen magnum the posterior part of the vermis and both paramedian lobules were exposed. Through this opening the entire cerebellum was removed by means of forceps and sucker.

Serial transverse sections show that all what is left of the cerebellum is part of the left flocculus (fig. 2). There is a necrosis extending into both restiform bodies, both brachia pontis, less into the brachia conjunctiva, presumably produced by the traction exerted on these structures during suction. The necrosis is limited to these fiber bundles and does not exceed their borders. The superficial part of the vestibular nuclei shows some glial proliferation, otherwise there are no alterations in the brain stem.

Red nucleus. Clearcut retrograde cellular changes are found bilaterally (fig. 2). They are most marked in the caudal third of the

nucleus, where more than one-third of all cells are changed. There is some predominance of affected cells medially. As more rostral levels are reached, the number of altered cells decreases by and by, and in the rostral third there are only scattered degenerating cells. The density of the dots in figure 2 gives an impression of the relative number of changed cells at the levels drawn. The inset in figure 2 shows a camera lucida drawing of the nucleus at the level of drawing 6. All changed cells are shown by open circles, all normal cells present by solid dots.

The retrograde changes are especially striking in the large cells of the nucleus (figs. 9 and 10), but also medium-sized cells are typically altered, showing tigrolysis and a nucleus displaced to the extreme periphery of the cell. Even some of the small cells are pathological, but the changes in these are less impressive.

The same cellular changes and a similar distribution of altered cells is found in 4 other animals subjected to total decerebellations (cat O 93, age at operation 15 days, killed 5 days later; cat O 120, age at operation 10 days, killed 5 days later; cat O 126, age at operation 10 days, killed 6 days later [fig. 11]; cat O 149, age at operation 10 days, killed 9 days later). In all of these animals only remnants of the flocculi and in two of one paraflocculus were left. In all of them there was some necrosis of fiber bundles in the cerebellar peduncles, and in three of them there was slight superficial damage to the vestibular nuclei.

The consistent occurrence of retrograde cellular changes in the red nuclei in these decerebellated animals permits the conclusion that some cells of this nucleus send their neurites to the cerebellum. These cells belong to all types, but a majority of them appears to be of the large type, only few of the small type, although it is possible that slighter changes

Fig. 2 Diagram of the findings in cat O 131. Below a section showing the maximal extent of the lesion. Simple hatchings indicate areas slightly altered, cross hatchings indicate necrotic areas. Above a series of drawings from equally spaced transverse Nissl-stained sections of the mesencephalon. The relative density of dots in the red nuclei gives an impression of the intensity of the changes at different levels. The inset to drawing 6 is a camera lucida drawing (checked under the microscope) of the left red nucleus at this level, showing all cells present, the normal ones black, those presenting typical retrograde changes white. See also figures 9 and 10.

in the latter have not been considered significant and have, therefore, been disregarded. It follows from the distribution of altered cells that the rubro-cerebellar fibers take origin

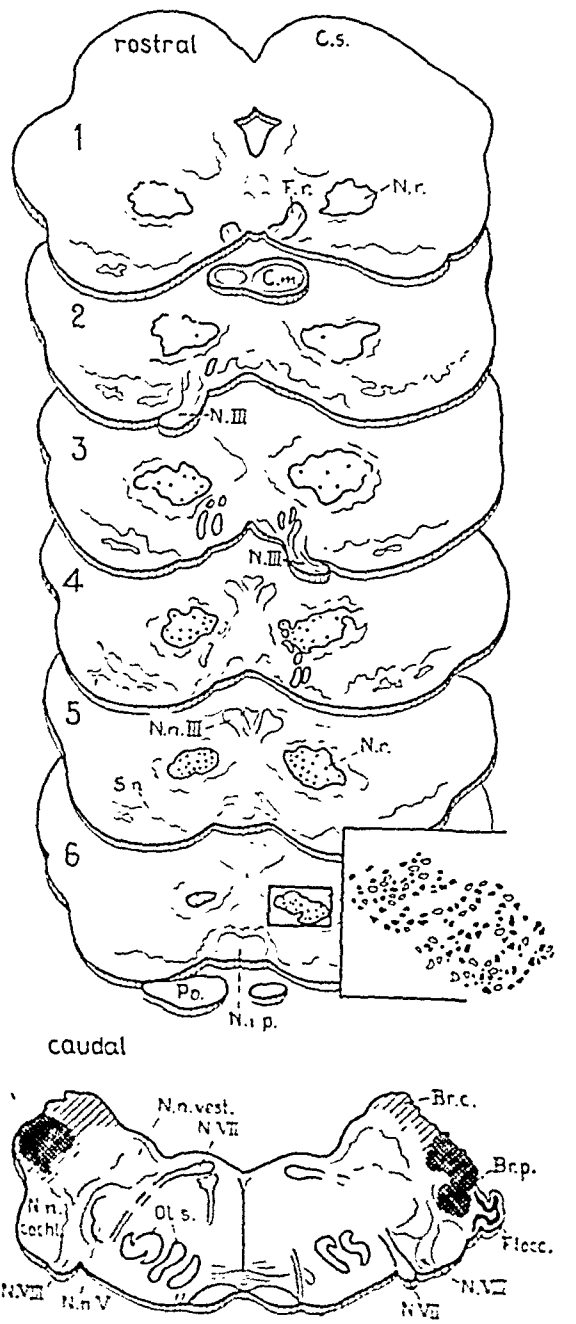


Figure 2

preponderantly from the caudal third of the red nucleus, with a certain overweight from its medial part. But even a few cells in the rostral third of the nucleus project on to the cerebellum.

The question may be raised whether the damage to the vestibular nuclei present in most of the decerebellated animals might be responsible for the cellular changes in the red nucleus. However, this damage is always superficial and very moderate, and, therefore, unlikely to give such marked and widely distributed cellular changes in the nucleus. Furthermore similar changes are observed in the red nucleus in other animals with an incomplete decerebellation without concomitant injury to the vestibular nuclei (vide infra). Although the material available does not exclude the possibility that the vestibular nuclei may receive some rubro-fugal fibers, we feel safe to conclude from our experiments that the cerebellum receives the neurites of the bulk of the changed cells in the red nucleus.

Further information of the rubro-cerebellar projection is obtained from cases with more circumscribed cerebellar lesions. In several animals the cerebellar lesions were limited to the vermis. In some of them it included also the fastigial nucleus. The lesions in 6 of these cases are shown in figure 3 and will be briefly described.

Cat O 105. (Age at operation 18 days, killed 5 days later.) The caudal two-thirds of the lobulus c_2 and the rostral half of the lobulus c_1 of Bolk have been removed by suction. There is a small bleeding in the right paramedian lobule, but no damage to the central white matter and the intra-cerebellar nuclei.

Cat O 123. (Age at operation 10 days, killed 5 days later.) The caudal two-thirds of the lobulus c_2, the entire lobulus c_1 (pyramis), more than the left half of the lobulus b (uvula) and some of the lobulus a (nodulus) have been removed. Slight damage has been made to the medialmost part of the left paramedian lobule, and the left fastigial nucleus is almost destroyed. A very small number of fibers from the most caudal part of the left dentate nucleus may possibly have been cut.

Cat O 133. (Age at operation 9 days, killed 5 days later.) Part of the lobulus c_1 (pyramis) and almost the entire lobulus b (uvula)

have been removed. There is a trifling injury to the left fastigial nucleus.

Cat O 138. (Age at operation 8 days, killed 4 days later.) The lobulus c_1 (pyramis) and the caudal third of the lobulus c_2 have been removed or destroyed. The injury to the latter lobule extends laterally into the right anso-paramedian lobule and in the midline has damaged to a moderate degree both fastigial nuclei.

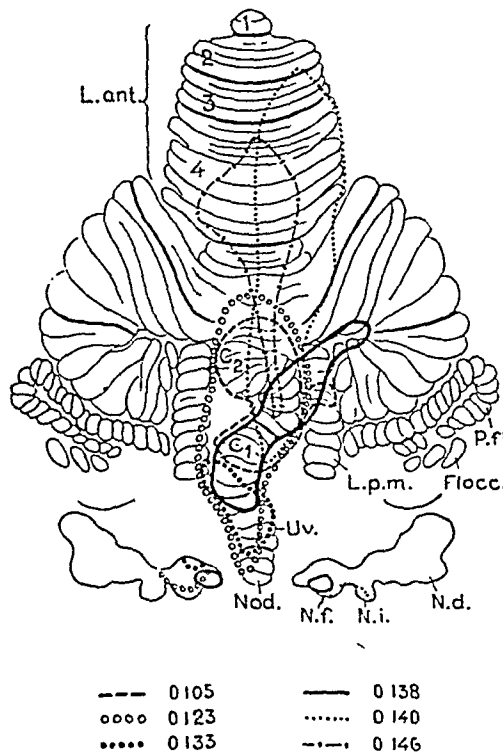


Fig. 3 Diagram showing in outlines on the unfolded cerebellar cortex the extent of the lesions in 6 cats in which chiefly parts of the vermis were removed. Abbreviations as in figure 1.

Cat O 140. (Age at operation 9 days, killed 5 days later.) The right half of the anterior lobe is almost completely removed. The lesion extends caudally into the lobulus c_2 and has also slightly encroached upon the lobulus c_1 (pyramis). There is slight affection of the right fastigial nucleus and the nucleus interpositus but no affection of the dentate. The right inferior colliculus is superficially damaged.

Cat O 146. (Age at operation 8 days, killed 5 days later.) The middle parts of the lobulus 4 (culmen) of the anterior lobe have been

removed. A more superficial lesion extends caudally near the midline as far as to the caudal end of the lobulus c_2. There is no damage to the central white matter and the nuclei.

In 5 of these cases as well as in some others with similar lesions no altered cells could be found in the red nuclei. We are inclined to conclude, therefore, that the parts of the cerebellum damaged in these animals, namely the anterior lobe, the middle and posterior parts of the vermis as well as the nucleus fastigii, do not participate in the rubro-cerebellar projection. It must be admitted, however, that this possibility cannot be definitely ruled out, since the absence of retrograde cellular changes is by itself not conclusive, particularly in view of the individual variations with regard to the speed of development of these changes, as mentioned before. On the other hand, the rather large number of such negative cases lends support to the conclusion mentioned.

In one case, cat 0 140 (fig. 3), altogether three typically changed cells were found in the red nuclei in all sections examined. Since a moderate involvement of the nucleus interpositus is the only principal difference between the lesion in this case and the others shown in figure 3, we are inclined to assume that a few fibers from the red nucleus terminate in the nucleus interpositus. This conclusion is supported by the findings in another case (cat O 132) illustrated in figure 4. This shows the lesions in 4 animals in which the cortex of a cerebellar hemisphere was more or less completely removed.

Cat O 104. (Age at operation 18 days, killed 8 days later.) The right paramedian lobule, the crus II of the ansiform lobule and part of the paraflocculus have been removed by means of knife and suction. The sagittal cut made to separate the paramedian lobule from the vermis has extended a little too far rostrally, separating the fastigial nucleus from the interpositus by a slit. The dentate nucleus is present, but owing to its close vicinity to the lesion of the paraflocculus there is some glial infiltration in its caudo-lateral pole.

Cat O 132. (Age at operation 9 days, killed 5 days later.) Most of the vermis, almost the entire right anso-paramedian lobule and part of the left ansiform lobule have been removed. The right nucleus fastigii and nucleus interpositus have been removed with only slight

glial infiltration in the neighboring parts of the right dentate. On the left there is partial but rather extensive damage to the nucleus fastigii and to the nucleus interpositus.

Cat O 147. (Age at operation 8 days, killed 5 days later. Parts of the left paramedian lobule and of the crus II have been removed with slight encroachment upon the paraflocculus. The left dentate nucleus is intact except for some glial infiltration and slighter nerve cell changes in the caudalmost part of its lateral pole.

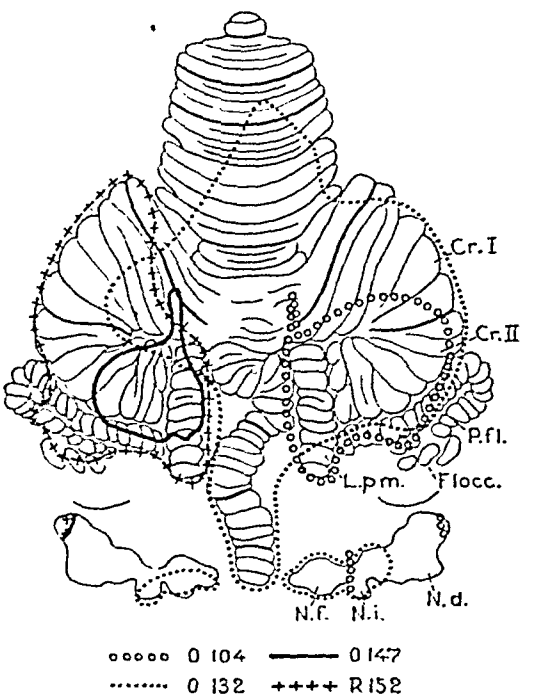


Fig. 4 Diagram showing in outlines on the unfolded cerebellar surface the extent of the lesions in 4 cats in which chiefly parts of the cerebellar hemispheres were removed. Abbreviations as in figure 1.

Cat R 152. (Age at operation 8 days, killed 5 days later.) Almost the entire left cerebellar hemisphere has been removed, including most of the paraflocculus. Only remnants of the left flocculus are present. The lateralmost pole of the left dentate nucleus shows some glial infiltration and some altered nerve cells, but there is no primary injury to the dentate.

In three of the cases shown in figure 4 (cats O 104, O 147 and R 152) no retrograde changed cells were present in the red nuclei, while in cat O 132 a moderate number of such cells was found (chiefly in the rostral part of the caudal third

of the nuclei). Since thus removal of extensive parts of the cortex of the ansoparamedian lobule and of parts of the paraflocculus is not followed by the occurrence of retrograde changed cells in the red nucleus, it appears likely that the rubro-cerebellar fibers do not reach the cerebellar cortex of the hemispheres. The presence of some altered cells in cat O 132 is reasonably explained by the extensive removal of the nuclei interpositi in addition to the cortical removal, a conclusion in agreement with that drawn from the findings in the cases shown in figure 3. The findings in another case (cat O 129) with removal of almost the entire vermis and rather extensive damage to the nuclei fastigii and interpositi are also in agreement with this. However, the number of altered cells following lesions including the nuclei interpositi is very moderate as compared with that seen consistently after total decerebellations. The following case provides an explanation of this discrepancy.

Cat O 119. Age at operation 8 days. Killed 4 days later (Figs. 5 and 12)

Lesion. By means of a knife a slit was made on the border between the vermis and the left hemisphere and the latter removed by suction. Transverse serial sections through the cerebellum and brain stem show that the entire left hemisphere, including the paraflocculus and flocculus, has been removed. There is some damage to the lateralmost folia of the lobulus 4 of the anterior lobe and slight involvement of the left side of the lobulus c_2 of the vermis. The left dentate is completely removed while there is a small remnant left of the nucleus interpositus. The fastigial nucleus is intact. A necrosis, presumably due to traction, extends into the brachium pontis on the left, but there is no affection of nuclei of the brain stem.

Red nucleus. Typically retrograde changed cells are found in both red nuclei (fig. 12). Their number is considerably less than in the cases with total decerebellations, but their localization within the nucleus is the same, i.e. they are found chiefly in its caudal third, decreasing in number as more rostral levels are reached. In the rostral third scarcely any altered cells are present. The changes are bilateral, but the number of altered cells on the right side, contralateral to the lesion, is about double of that on the left.

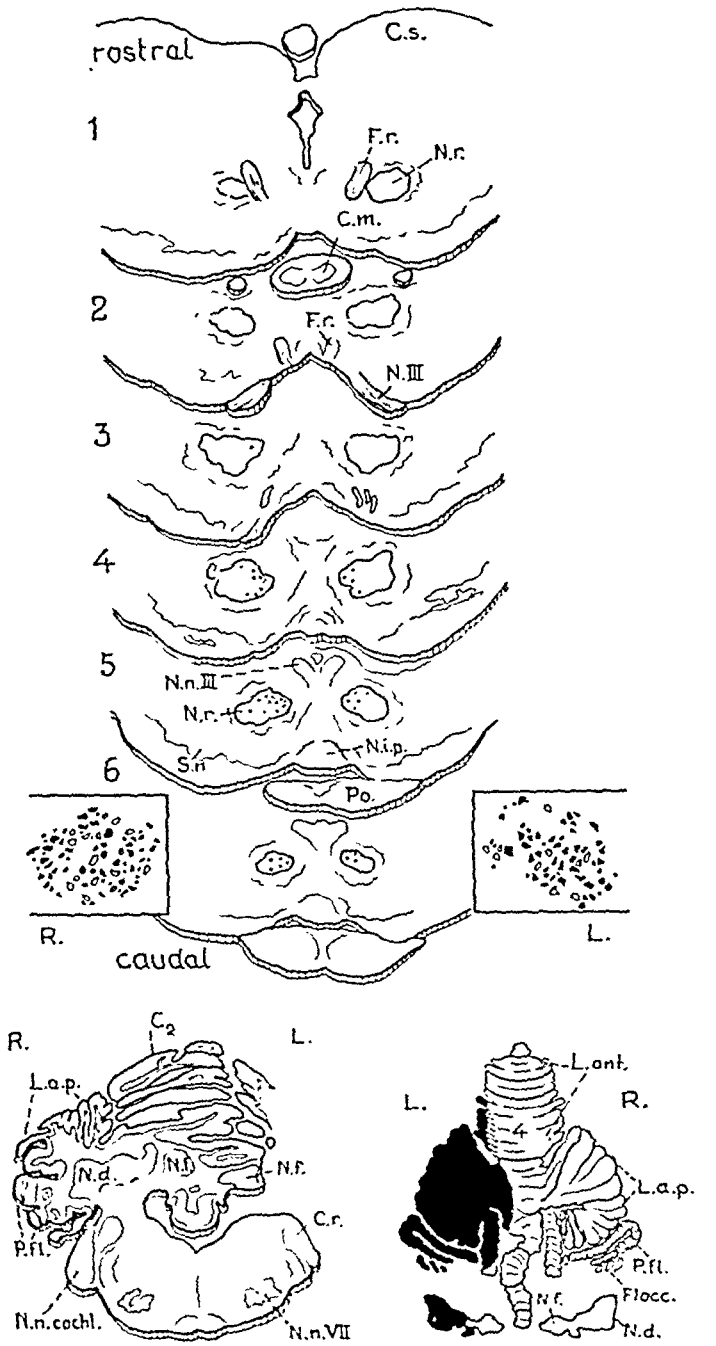


Fig. 5 Diagrammatic representation of the findings in cat O 119. Above a series of sections through the mesencephalon showing the distribution and relative density of retrograde changed cells in the red nuclei after the same principle as in figure 2. Below drawings showing the extent of the lesion. The parts of the cerebellum shown in black in the diagram of the unfolded cerebellar surface have been totally removed. Hatchings indicate slighter changes. Abbreviations as in figure 1.

The ample occurrence of altered cells in the red nucleus in this case must be ascribed to the removal of the dentate nucleus, since lesions involving only those parts of the cortex which have been removed in this case do not produce such changes.[1] The distribution of altered cells within the red nuclei is identical with that following total decerebellations, but their number is more restricted, as might be expected on account of the unilateral lesion. Although the method employed does not permit exact quantitative estimates, the changes in the latter case are so marked that they appear to approach half of those following decerebellations. The conclusion seems permissible, therefore, that the bulk of the rubro-cerebellar fibers terminates in the dentate nucleus. A much smaller proportion reaches the nucleus interpositus, while no such fibers have been traced to the cerebellar cortex and the fastigial nuclei. Whether there is any differential distribution within the dentate nucleus cannot be ascertained. The absence of changes in the red nucleus when the lateralmost pole of the dentate is slightly affected (cp. fig. 4) does not exclude that also this part may receive rubral fibers.

The bilateral occurrence of changes in the red nuclei in the last case makes clear that the rubro-cerebellar projection is crossed as well as uncrossed, and that the proportion of crossed fibers is considerably larger than that of uncrossed. The absence of changes in the red nuclei following removal of the vermis suggests that the crossing of the rubro-cerebellar fibers takes place in the brain stem, since fibers crossing in the cerebellum would have been interrupted in some of these cases where the lesion extends ventrally to the 4th ventricle.

(c) Supplementary observations. In an attempt to throw light on some of the questions raised by the findings reported above sections were examined from the brains of some cats subjected to experiments performed to elucidate primarily other problems.

[1] The limit between the nucleus interpositus and the dentate nucleus is not morphologically distinct. In the above account the border has been chosen in agreement with Jansen and Brodal ('40), a little more medially than indicated by Snider ('40).

On account of the somewhat diverging views expressed in the literature concerning the termination of the brachium conjunctivum fibers, silver impregnated frozen transverse sections through the mesencephalon were prepared from a cat (B. St. L. 50) in which the vermis and one cerebellar hemisphere had been removed 5 days before sacrifice, with the aim of establishing more precisely the termination than can possibly be done by the Marchi method employed by previous workers. In these sections the massively degenerated brachium conjunctivum could be easily followed through its decussation and into the contralateral red nucleus. Terminal, very fine degenerating fibers were abundant in the caudal part of the nucleus, and some degenerating terminal boutons were also seen. In the caudal third the majority of fine fibers in the nucleus were degenerating, only few fine fibers appearing normal (fig. 13). Degeneration was somewhat more marked ventro-medially than laterally. As more rostral levels were reached the number of degenerating terminal fibers showed a steady decrease, but even at a level corresponding to drawing 2 of figure 1, there was still a considerable number of fine fibers in degeneration, also here more conspicuous medially (fig. 14).

While a single case like this does not give information of details it permits the conclusion that the termination of brachium conjunctivum fibers is not limited to the caudal part of the nucleus, even if it is more abundant here than at rostral levels. There appears to be a similar distribution of termination of the afferent cerebellar fibers as of the cells of the red nucleus projecting on to the cerebellum. It was, however, not possible to detect in these silver preparations cells which could be identified as being in the state of retrograde degeneration (presumably because the animal was adult and had survived for 5 days only). The interesting question, whether the cerebello-rubral fibers establish synaptical contact with cells which project back to the cerebellum could, therefore, not be answered.

Since the rubro-spinal tract is known to take origin from the caudal part of the red nucleus, it was of interest to compare the distribution of retrograde cells following decerebellations with the distribution following interruption of the rubro-spinal tract. Two cats (O 141 and O 144) in which transverse lesions had been made in the spinal cord at the level of C_3 at the age of 11 days and which had been sacrificed 5

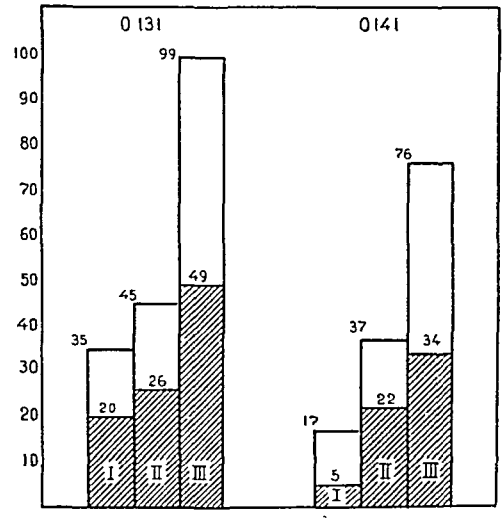


Fig. 6 Diagram showing the numbers of retrograde changed (hatchings) and normal (white) cells in three caudal sections from the red nucleus in a decerebellated cat (O 131) and in a cat in which the rubro-spinal tract had been transected at the level of C_3 (O 141). Column I represents the most caudal of the sections of the series passing through the red nucleus; between sections I and II and III there is an interval of 4 sections which were not mounted. Since the first section cuts the nucleus more caudally in cat O 141 than in O 131 cell numbers are less in all sections from this animal than in O 131. Cp. text.

days later, were used for this purpose. Microscopical examination showed that in both of them the lateral funiculus had been completely transected. The retrograde changes in the contralateral red nucleus involved large, medium-sized and small cells and were most abundant in the caudal third, decreasing by and by in a rostral direction. The altered cells were, therefore, found at the same levels of the nucleus as the changes following decerebellation, but there was a tendency for the affected cells to be located laterally.

On account of the similar distribution of the cells giving origin to cerebellar and spinal fibers, and in view of the statement by Cajal ('09, vol. I, p. 255) that some cells of the red nucleus have neurites which dichotomize close to the perikaryon, the possibility must be considered that the same cells may project to the cerebellum and to the spinal cord. The normal and retrograde changed cells in one case of spinal cord lesion (cat O 141) and in one case of decerebellation (cat O 131) were, therefore, counted in the three most caudal sections of each series (after having been drawn in a projection apparatus and controlled under the microscope). Only cells representing the fully developed picture of retrograde changes were considered as significant, all other cells were counted as normal ones. Tangentially cut cells without a nucleus were discarded.

The diagram of figure 6 shows the numbers of typical retrograde cells (hatchings) and of normal cells (white) in the three sections from the two cases, column I representing the most caudal section. As will be seen, in the decerebellated animal (O 131) in the two caudalmost sections more than half of the cells are altered, in the spinal animal this is the case only at level II. In sections rostral to those represented the proportion of altered cells decreases by and by in both cases. (The inset in fig. 2 is from the section following rostral to section III here.) When the three levels considered are taken together, they contain in the decerebellated animal 179 cells, 95 of which are altered, i.e. 53% of the cells present retrograde changes. In the animal with transection of the rubrospinal tract at C_3, there are altogether in the three sections 130 cells, 61 of which are pathological, i.e. 47%. These findings will be discussed below.

DISCUSSION

The changed cells of the red nucleus described and illustrated in this paper present all those features which are considered as evidence of retrograde cellular alterations, i.e.

changes occurring in the perikarya of nerve cells following transection of their neurites. Even if such changes, as far as we are aware, have not been observed in the red nucleus following lesions of the cerebellum by previous authors we feel convinced that their presence in the experimental animals betrays the existence of true rubro-cerebellar fibers, terminating chiefly in the dentate, to a small extent also in the nucleus interpositus. This interpretation is supported by the very close similarity between the celllular changes described and those observed in other nuclei, the cerebellar projection of which has been substantiated also by other methods. Furthermore, exactly similar changes occur in the cells of the red nucleus following lesions of the brain stem or upper part of the cervical cord involving the rubro-spinal tract, as we have been able to verify ourselves.

It should be emphasized, however, that the lack of retrograde changes in a nucleus does not warrant the conclusion that it does not contribute fibers to a particular fiber bundle which has been transected. Our experiments, therefore, indicate the minimum of possibly existing connections, the more so since only definitely altered cells, presenting a clearcut picture of retrograde changes, have been taken into account. We are not entitled to exclude definitely that a few of the rubro-cerebellar fibers may terminate in the fastigial nucleus or in the cerebellar cortex, even if the consistently negative findings in a fairly large number of cases make this unlikely. In spite of the limitations inherent in the method employed here, it is nevertheless the best suited at present. Since the rubro-cerebellar connections are crossed as well as uncrossed, a study of the later stages presenting cell loss would be less satisfactory on account of the lack of a normal control side.

It is of some interest to compare our findings with observations reported by some early authors who have advocated the presence of rubro-cerebellar connections. Forel in 1881 was the first to describe an atrophy of the posterior third of the red nucleus in a rabbit in which he had damaged the brachium conjunctivum and Gudden (1882) and Vejas (1885)

observed the same following extirpation of one cerebellar hemisphere. Mahaim (1894) following transection of the superior cerebellar peduncle in a one day old rabbit found 4½ months later an almost complete disappearance of nerve cells in the caudal third of the contralateral red nucleus, and a smaller loss of cells in its middle third. Some of the cells in the altered parts of the nucleus were diminished in size. All these early authors employed the original Gudden method, operating their animals immediately after birth or in the course of the first day and kept them for several weeks or months.

The findings made by these authors were not considered valid by subsequent workers, and the objections were made that there were accidental lesions, or e.g. by Cajal, that the changes were transneuronal. The observations made in the present study leave no doubt, however, that these authors were right in concluding that fibers from the posterior third of the red nucleus pass to the cerebellum, even if their assumption that the brachium conjunctivum is composed of descending fibers only has been disproved.

The distribution of retrograde changed cells in the red nucleus observed in the present investigation is practically identical with the sites of cell loss reported by Mahaim (1894) in his careful study. However, the smaller homolateral contribution of rubro-cerebellar fibers demonstrated here has been overlooked by the early authors, since they examined their animals after a lapse of time when all altered cells had disappeared. It is in agreement with our findings that Mahaim did not find alterations in the nucleus minimus.

A comparison between the rubro-cerebellar projection as determined here and the cerebello-rubral connections studied by numerous previous authors is of some interest. On most points these two fiber systems betray striking similarities.[2]

[2] It has been assumed here that the rubro-cerebellar fibers enter the cerebellum via the brachium conjunctivum. Our experiments, however, do not permit us to state this with certainty, and we are not entitled to exclude that the fibers may enter via the restiform body or the brachium pontis, although this appears less likely.

Almost all authors are in agreement with regard to the complete crossing of the fibers of the brachium conjunctivum (for example Klimoff, 1899; Allen, '24; Mussen, '27; Gerebtzoff, '36). Probst ('02), however, has suggested that there may be also some uncrossed fibers to the red nucleus, but he derives them from the fastigial nucleus. With regard to the crossing of fibers, there may, therefore, be a difference between the rubro-cerebellar and the cerebello-rubral projections. It is possible, however, that a minor homolateral contingent of cerebello-rubral fibers has been disregarded by the workers who have employed the Marchi method. Our case of hemidecerebellation is suggestive of terminal degeneration also in the homolateral nucleus.

While all authors agree that the dentate nucleus is an important source of the fibers of the brachium conjunctivum, others have adduced evidence that fibers from the nucleus interpositus, corresponding to the globose and emboliform nuclei in man, also take part in the projection (Klimoff, 1899; Allen, '24; Mussen, '27; Winkler, '27; Gerebtzoff, '36; Jansen and Jansen). Some even maintain that a small contribution comes from the fastigial nucleus (Probst, '02; Preisig, '04). We have not been able to demonstrate rubro-fastigial fibers (although their presence cannot be excluded), but our study makes clear that the bulk of the rubro-cerebellar fibers reach the dentate while a small proportion terminates in the nucleus interpositus. Apart from the more moderate number of fibers related to the nucleus interpositus in the rubro-cerebellar than in the cerebello-rubral projection there appears thus to be a correspondence between these fiber systems with regard to their relation to the intra-cerebellar nuclei. Of relevance in this connection are Hassler's ('50) findings in human material. According to Hassler the brachium conjunctivum fibers originating in the nucleus emboliformis reach the central nucleus of the thalamus (centre médian of Luys), the fibers from the parvi-cellular part of the dentate pass to the ventral thalamic nucleus, while only those from the magno-cellular, phylogenetically oldest part of the dentate

terminate in the red nucleus, in its caudal magno-cellular (which is small in man) as well as in its parvi-cellular part. Authors working experimentally who have considered this problem, have expressed the opinion that the fibers from the nucleus interpositus and those from the dentate reach the same parts of the red nucleus (Allen, '24; Mussen, '27). It should be recalled, however, that the Marchi method, employed by these authors, is not well suited to determine the exact termination of degenerating fibers, and the fibers from the nucleus interpositus may chiefly be fibers passing by. The study of cell loss in Hassler's cases makes possible more definite conclusions. It would, therefore, not be improbable that conditions also in the cat are in principle as Hassler has described them in man. If so, the parallelism between the cerebello-rubral and rubro-cerebellar projections would be very close with regard to their relation to the intra-cerebellar nuclei. However, it appears from the studies of Jansen and Jansen, Jr. (in press) that although the projections of the dentate and the nucleus interpositus are not identical, the conditions in the cat appear not to be as described for man by Hassler ('50). Phylogenetical differences will, of course, have to be taken into account when this problem is to be studied.

The rubro-cerebellar fibers, as has been seen, take origin chiefly from the caudal, magno-cellular part of the red nucleus, but scattered fibers come also from the rostral parvicellular part. This area of origin coincides with that to which most authors have traced cerebello-fugal brachium conjunctivum fibers, be they all derived from the dentate or some also from the nucleus interpositus. The distribution of terminal degeneration within the red nucleus following removal of one-half of the cerebellum confirms this parallelism. A two-way connection between the dentate and the red nucleus, therefore, exists, and it appears likely that a considerable number of cells in the caudal part of the red nucleus which project on to the dentate may be influenced by impulses from this nucleus. Whether the cerebello-rubral fibers establish synaptical contact with the perikarya of the rubro-cerebellar fibers, we have, however, not been able to decide.

A final question to be considered is the relationship between the rubro-spinal and the rubro-cerebellar projections. The rubro-spinal tract has been shown to take origin chiefly from the caudal magno-cellular part of the red nucleus (e.g. by Preisig, '04; von Monakow, '09; and others), and our series with spinal cord lesions confirm this. They also show that some fibers come from the parvi-cellular part, in decreasing numbers as more rostral levels are reached, and that not only large cells but also medium-sized and small cells give origin to some rubro-spinal fibers as mentioned by von Monakow ('09). The rubro-spinal and the rubro-cerebellar projections, therefore, appear to be derived from the same areas of the red nucleus, apart from a slight difference insofar as the cerebellar fibers take origin with a certain preponderance from the medio-ventral parts, the spinal fibers more from the lateral part of the red nucleus. Since the number of altered cells in the red nucleus following decerebellations as well as following high level spinal cord lesions is remarkably large, the possibility suggests itself that some of the cells may give origin, by means of a branching neurite, to both types of fibers, a possibility supported by Cajal's Golgi studies. The results of quantitative studies performed to test this possibility are represented in the diagram of figure 6. Fifty-three per cent of the cells in the caudal part of the red nucleus show changes following decerebellation, 47% are changed following a lesion at C_3 of the spinal cord. Even if thus these figures together account for all cells in the caudal part of the nucleus, the values obtained are certainly too low. First, only cells showing fully developed retrograde alterations have been taken into account, and it is almost certain that in both cases some cells with slighter changes, representing earlier phases of their development, have been disregarded. Second, a transection of the rubro-spinal tract at the level of C_3 can scarcely be imagined to have cut all rubro-spinal fibers and will at least have spared possible rubro-bulbar fibers (von Monakow, '09).

The findings, therefore, strongly suggest that some cells of the red nucleus project by means of a branching neurite to the dentate nucleus as well as to the spinal cord.[3] It is of interest in this connection to recall that some of the cerebello-rubral fibers project caudally by means of a collateral, forming the brachium conjunctivum descendens. Even if it is as yet unknown to what extent the terminations of these two descending pathways may be identical, their existence furnishes another example of the parallelism in the organization of the cerebello-rubral and rubro-cerebellar connections.

The functional significance of the pathway demonstrated in this study remains a subject for physiological studies. We shall refrain from such speculations which may be made at our present state of knowledge. Suffice it to draw attention to the fact that this cerebello-rubro-cerebellar pathway is another example of systems made up of fibers conducting in both directions, organized so as to make possible nervous activity in closed neuronal circuits and representing the structural basis of feed-back mechanisms. Two-way connections of this type appear, indeed, to be more common than hitherto assumed.

SUMMARY

The existence of rubro-cerebellar connections, advocated by some early authors, has not been generally admitted by subsequent investigators. By the use of the modified Gudden method it has been possible to demonstrate these connections.

An account of the normal architecture and topography of the red nucleus in the cat is given. There seems to be no

[3] If the assumption set forth here is correct, it may seem strange that these cells react equally well with retrograde changes to a transection of either of their two neuritic branches, since the preservation of proximal collaterals has been frequently adduced to explain a lack of retrograde changes in nerve cells following transection of their neurites. However, this protecting influence of preserved collaterals, as far as we are aware, has never been proved. On the other hand it is well known that different nerve cells exhibit marked differences with regard to their reaction to a transection of their neurites.

reason to distinguish other subdivisions than a caudal magnocellular and a rostral parvi-cellular part, fusing without sharp transitions. Even in the caudal part there are some small cells, and scattered large cells occur in the rostral part.

Following total decerebellations in 6 cats aged 8–18 days and killed 5–9 days later typical retrograde changed cells occur in the red nucleus. All types of cells are affected, most of them being found in the caudal pole, where more than half the number of cells present are affected, but also in the rostral part of the nucleus scattered altered cells occur. It is concluded that these cells send their neurites to the cerebellum.

Following lesions restricted to the cerebellar vermis and the nucleus fastigii or to the cortex of the hemispheres no changes are found in the red nucleus. When the nucleus interpositus is damaged there are a few altered cells, and when the dentate nucleus is included in the lesion there is an affection of about half as many cells as when the entire cerebellum is removed. Considerably more cells are affected in the contralateral nucleus than in the homolateral. It is concluded that the rubro-cerebellar projection is chiefly crossed, to a less extent uncrossed, and that the bulk of its fibers reach the dentate nucleus, a small contingent the nucleus interpositus.

The distribution of the cells of origin of the rubro-cerebellar projection coincides very closely with the terminal area of the cerebello-rubral fibers as determined by the method of terminal degeneration. The rubro-spinal tract also takes origin from the same part of the nucleus. Quantitative estimates make probable that there are in the caudal part of the red nucleus a certain number of cells which by means of a dichotomizing neurite projects on to the cerebellum as well as to the spinal cord. The rubro-cerebellar and the cerebello-rubral fibers establish a two-way connection between the dentate nucleus and the red nucleus.

LITERATURE CITED

ALLEN, W. F. 1924 Distribution of the fibers originating from the different basal cerebellar nuclei. J. Comp. Neur., *36:* 399–439.

BRODAL, A. 1939 Experimentelle Untersuchungen über retrograde Zellveränderungen in der unteren Olive nach Läsionen des Kleinhirns. Zschr. f. d. ges. Neur. und Psychiat., *166:* 646–704.

———— 1940 Modification of Gudden method for study of cerebral localization. Arch. Neur. and Psychiat., *43:* 46–58.

———— 1941 Die Verbindungen des Nucleus cuneatus externus mit dem Kleinhirn beim Kaninchen und bei der Katze. Zschr. f. d. ges. Neur. und Psychiat., *171:* 167–199.

———— 1943 The cerebellar connections of the nucleus reticularis lateralis (nucleus funiculi lateralis) in rabbit and cat. Experimental investigations. Acta Psychiat. et Neur. Kbh., *18:* 171–233.

———— 1952 Experimental demonstration of cerebellar connexions from the peri-hypoglossal nuclei (nucleus intercalatus, nucleus praepositus hypoglossi and nucleus of Roller) in the cat. J. Anat., *86:* 110–129.

———— 1953 Reticulo-cerebellar connections in the cat. An experimental study. J. Comp. Neur., *98:* 113–153.

BRODAL, A., AND J. JANSEN 1946 The ponto-cerebellar projection in the rabbit and cat. Experimental investigations. J. Comp. Neur., *84:* 31–118.

CAJAL, S. RAMÓN Y 1909 Histologie du système nerveux de l'homme et des vertébrés. Paris, Maloine.

DAVENPORT, H. A., AND S. W. RANSON 1930 The red nucleus and adjacent cell groups. A topographic study in the cat and in the rabbit. Arch. Neur. and Psychiat., *24:* 257–266.

FOREL, A. 1881 Einige hirnanatomische Untersuchungen. Tagebl. 54. Vers. Dtsch. Naturforsch. u. Ärzte, Salzburg, p. 185. Quoted from Vejas, 1885 and Mahaim, 1894.

GERBERTZOFF, M. A. 1936 Le pédoncule cérébelleux supérieur et les terminaisons réelles de la voie cérébello-thalamique. Mém. Acad. Roy. Méd. Belg., *25:* 1–58.

GLEES, P. 1946 Terminal degeneration within the central nervous systems as studied by a new silver method. J. Neuropath. and Exp. Neur., *5:* 54–59.

GUDDEN, B. VON 1882 Über die Verbindungsbahnen des kleinen Gehirns. Tagebl. 55. Vers. Dtsch. Naturforsch. u. Ärzte, Eisenach, pp. 303–305. Quoted from Vejas, 1885 and Mahaim, 1894.

HASSLER, R. 1950 Über Kleinhirnprojektionen zum Mittelhirn und Thalamus beim Menschen. Dtsch. Zschr. Nervenheilk., *163:* 629–671.

HATSCHEK, R. 1907 Zur vergleichenden Anatomie des Nucleus ruber tegmenti. Arb. a. d. neurol. Inst. a. d. Wiener Univ., *15:* 89–123. Quoted from v. Monakow, 1909.

JANSEN, J., AND A. BRODAL 1940 Experimental studies on the intrinsic fibers of the cerebellum. II. The cortico-nuclear projection. J. Comp. Neur., *73:* 267–321.

JANSEN, J., AND J. JANSEN, JR. On the efferent fibers of the cerebellar nuclei in the cat. J. Comp. Neur. (in press).

KLIMOFF, J. 1899 Über die Leitungsbahnen des Kleinhirns. Arch. f. Anat. u. Entw. gesch., Anat. Abt., pp. 11–27.

MAHAIM, A. 1894 Recherches sur la structure anatomique du noyau rouge et ses connexious avec le pédoncule cérébelleux supérieur. Mémoires Couronnés, Acad. Roy. Med. Belg., *13:* 1–44.

MONAKOW, C. VON 1909 Der rote Kern, die Haube und die Regio hypothalamica bei einigen Säugetieren und beim Menschen. I Teil: Anatomisches und Experimentelles. Arb. a. d. hirnanat. Inst. in Zürich, *3:* 51–267.

MUSSEN, A. T. 1927 Experimental investigations on the cerebellum. Brain, *50:* 313–349.

PREISIG, H. 1904 Le noyau rouge et le pédoncule cérébelleux supérieur. J. f. Psychol. u. Neur., *3:* 215–230.

PROBST, M. 1902 Zur Anatomie und Physiologie des Kleinhirns. Arch. f. Psychiat., *35:* 692–777.

SNIDER, R. S. 1940 Morphology of the cerebellar nuclei in the rabbit and the cat. J. Comp. Neur., *72:* 399–415.

VEJAS, P. 1885 Experimentelle Beiträge zur Kenntnis der Verbindungsbahnen des Kleinhirns und des Verlaufs der Funiculi graciles und cuneati. Arch. f. Psychiat., *16:* 200–214.

WINKLER, C. 1927 Manuel de Neurologie. Tome I: L'anatomie du système nerveux, 3ème partie, pp. 1–377. Haarlem, de Erven F. Bohn.

WINKLER, C., AND A. POTTER 1914 An anatomical guide to experimental researches on the cat's brain. Amsterdam, W. Versluys.

PLATE

PLATE 1

EXPLANATION OF FIGURES

7 Camera lucida drawings of Nissl-stained large, medium-sized and small cells from the normal red nucleus in cats two to three weeks old (to the left) and of the same types of cells in the fully developed stage of retrograde changes following decerebellation (to the right). × 600.

8 Photomicrograph from the caudal part of the red nucleus in a normal cat, 18 days old. Chiefly large, but also medium-sized and small cells are present. × 260.

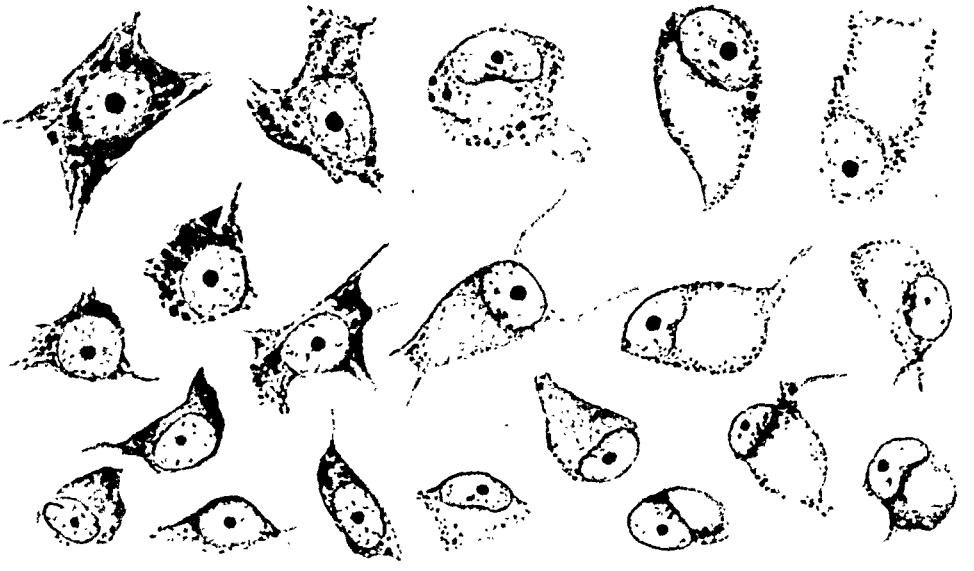
9 Photomicrograph from the caudal part of the red nucleus in cat (O 131), decerebellated 10 days old, killed 8 days later. Numerous cells present typical retrograde changes. × 65.

10 Photomicrograph from the red nucleus as shown in figure 9. Most cells present are pathological. × 260.

11 Photomicrograph from the caudal part of the red nucleus in another cat (O 126), decerebellated 10 days old, killed 6 days later. × 260.

12 Photomicrograph showing retrograde changed cells in the caudal part of the right red nucleus in cat O 119, following removal of the left cerebellar hemisphere and dentate nucleus. The animal was operated on 8 days old and killed 4 days later. × 260.

13 and 14 Photomicrographs of silver impregnated transverse sections (Glees' method) from the red nucleus of an adult cat, killed 5 days after an extirpation of the contralateral cerebellar hemisphere. More abundant preterminal and terminal degenerating fibers (some of them indicated by arrows) in the caudal part of the nucleus (13) than in the rostral part (14). × 730.



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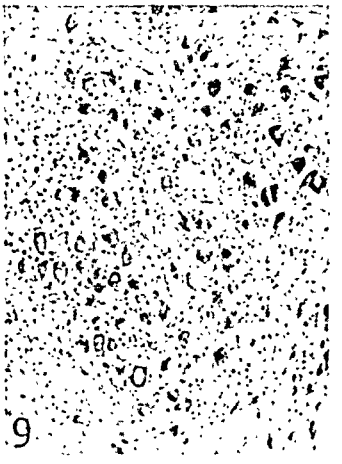

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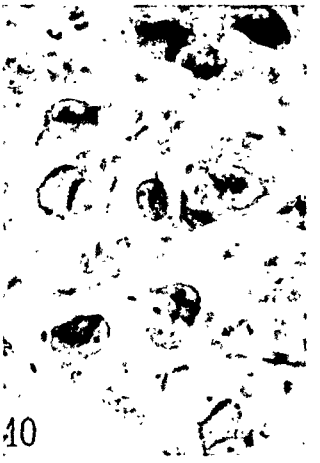

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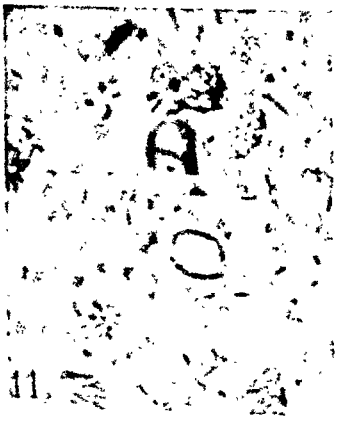

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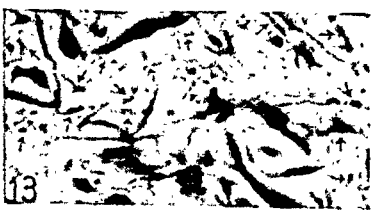

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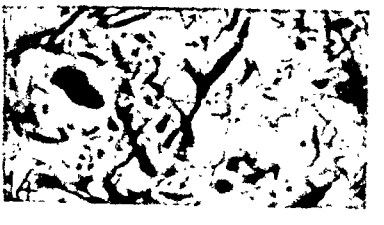

14

THE EFFECT OF PERIPHERAL NERVE SECTION ON SOME METABOLIC RESPONSES OF BROWN ADIPOSE TISSUE IN MICE[1]

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NINETEEN FIGURES

In the years since Schoenheimer ('42) demonstrated that the lipids of adipose tissue undergo constant metabolic turnover, considerable interest has developed in the factors which control the deposition and mobilization of lipid. While various hormones have been shown to have an effect upon adipose tissue (Fawcett and Jones, '49; Baker, '52), relatively little attention has been paid in recent years to the influence of the nervous system on adipose tissue even though detailed experimental studies on this subject are to be found in the European literature of a previous decade (Hausberger, '34, '35, '37, '39; Hausberger and Gujot, '37; Beznäk and Hasch, '37; Cedrangolo, '37; Kuré, Oi and Okinaka, '37). The present paper again directs attention to the nervous system as an important factor in the regulation of adipose tissue metabolism. The interscapular brown adipose tissue of mice and rats was examined for the presence of nerve fibers, and the effect of nerve section on the glycogen and lipid content of the interscapular fat bodies of mice was studied by both histochemical staining methods and quantitative chemical analysis. The results confirm and extend the observations of Hausberger ('34) who first demonstrated a transient deposition of glycogen and a lasting increase of lipid in denervated brown fat.

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[2] Jeffries Wyman Scholar of Harvard University 1951–53.
[3] John and Mary R. Markle Scholar in Medical Science.

MATERIAL AND METHODS

Two rats of the Hisaw strain and approximately 60 adult mice of the Swiss albino and Ce strains were used. Litter mate mice of the same sex were used for each experiment but in the entire study the numbers of males and females used were approximately equal. The animals were maintained on a diet of Purina rat chow and water *ad libitum.*

All observations on denervated adipose tissue were made on the interscapular brown fat, which is particularly suitable for such studies because it occurs as a pair of discrete fat bodies symmetrically placed on either side of the midline. Each member of the pair possesses an independent nerve supply. Thus the nerves to one side may be interrupted while the other side is maintained intact as a control. A sham operation was performed routinely on the control side. The operation produces minimal bleeding and negligible mortality.

For glycogen analyses the fat bodies were weighed on a torsion balance, digested with hot 30% KOH and the glycogen precipitated with 95% ethanol. Glycogen was measured colorimetrically in aqueous solution with the anthrone reagent according to the directions of Illingworth and Russell ('51).

Lipid analyses were performed by weighing one half of each member of the paired fat bodies, grinding with sand and extracting three times with ether. After removal of the ether and water the "ether-extractable lipid" was weighed. The other half of each fat body was dried to constant weight for analysis of water concentration. Lipid data are expressed as "mg lipid per mg fat-free dry tissue."

In addition, glycogen and fat were studied by histochemical means in tissue sections. Glycogen was stained by the periodic acid-Schiff technique in 5 μ paraffin sections after fixation of the tissue in cold alcohol-formalin-picric acid solution (Rossman's fluid). This method is more sensitive for glycogen in adipose tissue than other techniques, as illustrated previously (Fawcett, '48, figs. 1–3). Frozen sections of formalin-fixed tissue embedded in gelatin were stained for fat with

sudan black B. Additional paraffin sections were stained with haematoxylin and eosin.

The nerve supply to the brown fat body was demonstrated with protargol silver in 5 μ paraffin sections after fixation of the tissues in alcohol-formalin-acetic acid solution (Bodian, '37).

RESULTS

Innervation of the interscapular brown fat body. Six fairly large nerves enter each brown fat body at its ventro-medial surface (fig. 1) and run in the interlobular connective tissue septa (figs. 2, 6). Smaller branches of these nerves are found along the major interlobular blood vessels (figs. 4, 5). In addition, numerous fine nerve fibers leave the septa (fig. 6) to run a tortuous course among the fat cells as described in white fat by Boeke ('33) and in brown fat by Hausberger ('34). The number of fibers demonstrable in sections only 5 μ thick is surprisingly high. Many of these nerves do not appear to be associated with blood vessels but terminate as delicate fibers in direct contact with adipose cells (figs. 7 to 10). The fact that fibers of widely varying diameters are found in the large nerves entering the fat bodies (fig. 1) suggests that these are mixed nerves carrying fibers of more than one type. The location of the cell bodies of these nerve fibers and the direction of nerve impulse conduction within them are unknown.

Effect of denervation on glycogen content. Table 1 contains comparative data on glycogen concentrations in interscapular fat bodies from mice fasted 48 hours, denervated unilaterally, then refed *ad libitum* for 24 hours, and also in interscapular fat bodies from a group of animals similarly fasted and refed but not operated upon. As reported in previous qualitative histochemical studies, some glycogen may be deposited nocturnally in the fat bodies of normal mice, and deposition of considerable amounts of glycogen can be induced by various experimental procedures (Fawcett, '52). In table 1 quantitative data are presented for glycogen deposited during refeeding after a 48-hour fast. In the unoperated control ani-

mals (Group B) approximately the same amount of glycogen was deposited in the right and left interscapular fat bodies. On the other hand in the operated animals (Group A) a significantly greater amount of glycogen was deposited in the denervated left fat body than in the innervated right fat body. The increased glycogen deposition in recently de-

TABLE 1

Effect of denervation on concentration of glycogen in the interscapular brown fat

Mice of group A were fasted for 48 hours and their interscapular brown fat bodies were denervated on the left side. Postoperatively the mice were refed *ad libitum* for 24 hours. The fat bodies were then removed and analyzed for glycogen concentration. Mice of group B were similarly fasted and refed but were not operated upon.

	NO. MICE	WET WT.		GLYCOGEN		MEAN DIFF. IN GLYCOGEN CONC. (M.)
		Lt.	Rt.	Lt.	Rt.	
		mg		*mg %*		
Group A						
Lt. side denerv.	6	34.2 (26–54)	32.0 (22–54)	416 (177–715)	237 (101–355)	+ 40.4% (S.E. = 7.6)
Group B						
Non-op. controls	6	57.4 (33–73)	48.6 (30–58)	381 (65–681)	340 (72–653)	+ 7.8% (S.E. = 6.4) $P < 0.01$ (highly significant)

The mean difference (M.) and the standard error of the mean (S.E.) are based on % differences in glycogen concentrations between right and left halves of the brown fat body in individual animals. The values for mean and standard error comparing group A (operated) with group B (non-operated) were used for calculation of "t" values according to the formula $t = \frac{M_A - M_B}{\sqrt{(S.E._A)^2 + (S.E._B)^2}}$. P was derived from standard tables relating "t" and "n."

nervated adipose tissue was also apparent in histological preparations stained with the periodic acid-Schiff reaction (figs. 12, 13).

Effect of prolonged denervation on lipid content. Table 2 presents comparative values for lipid concentration in denervated and innervated brown adipose tissue. In the operated animals of this experiment (Group A) the nerves to one

of the paired interscapular fat bodies were sectioned and their proximal ends avulsed in order to prevent regeneration. The associated spinal ganglion was often avulsed with the proximal segment of the nerve. Postoperatively the animals were allowed a normal diet *ad libitum* for 30 days, at the end of which time they were killed, and both the denervated and nondenervated fat bodies of each animal were analyzed separately for total lipid.

TABLE 2

Effect of denervation on concentration of lipid in the interscapular brown fat

The interscapular brown fat bodies of mice in group A were denervated on the left side. Postoperatively the mice were fed a normal diet *ad libitum* for 30 days. The fat bodies were then removed and analyzed for lipid concentration. Mice of group B were similarly fed but were not operated upon.

	NO. MICE	WET WT.		LIPID WT./FAT-FREE DRY WT.		MEAN DIFF IN LIPID CONC.
		Lt.	Rt.	Lt.	Rt.	
		mg				
Group A						
Lt. side denerv.	6	57.0 (29–104)	34.9 (17–61)	1.62 (1.1–2.6)	1.02 (.4–1.6)	+ 39.1% (S.E. = 11.8)
Group B						
Non-op. controls	6	28.0 (25–34)	27.5 (22–35)	1.17 (.9–1.4)	1.19 (1.0–1.3)	— 2.0% (S.E. = 6.0) P = 0.01

Comparing right and left interscapular fat bodies of individual mice of the unoperated control group (Group B) there was no significant difference in lipid concentration. In the operated animals (Group A) the denervated left fat body was consistently heavier than the normal right side. This difference in weight was due to a significant increase in the quantity of ether-soluble lipid per unit of fat-free dry weight (table 2). The quantitative data were supplemented by examination of stained paraffin sections from control and denervated fat bodies prepared 4 and 30 days postoperatively. These sections revealed an increased size of lipid vacuoles,

an appearance consistent with an increase in lipid volume per cell (figs. 11, 14 to 16).

Effect of reduced temperature plus food deprivation on the acute mobilization of lipid. Mice were divided into three experimental groups as follows: (1) normal controls; (2) mice maintained at 5°C. without food until death; (3) mice which had the fat body of one side denervated and then, following recovery from anesthesia, maintained at 5°C. without food until death. The fat bodies were examined grossly and frozen sections were stained for lipid with sudan black B. Average survival time of mice deprived of food and kept in the cold (Groups 2 and 3) was 7 hours with a spread of 5 to 15 hours. There was no difference in survival time of unoperated animals (Group 2) and operated animals (Group 3) kept under these conditions. The fat bodies of control mice were plump and of the usual light reddish-brown color, while those of fasted, unoperated animals kept in the cold were small and very much darker as reported previously by Selye and Timiras ('49). The nondenervated fat bodies in Group 3 were likewise small and dark red, while the denervated fat bodies were larger and more pale in color like those of normal mice. Microscopically, the brown fat cells of intact mice fasted in the cold (Group 2) showed an almost complete absence of lipid droplets in contrast to the lipid-laden fat cells in normal mice. In operated animals fasted in the cold (Group 3) the innervated fat bodies were depleted of lipid while the denervated fat bodies retained their lipid (figs. 17 to 19). Thus, while lipid is rapidly withdrawn from the innervated fat body, it is not readily mobilized from a denervated fat body even during fatal exposure to low temperature plus food deprivation. This difference in fat mobilization from the two sides is already pronounced within 6 hours after denervation.

DISCUSSION

Brown fat contains a combination of enzymes necessary for the synthesis of glycogen from glucose comparable with

the enzymes found in muscle (Creasey and Gray, '52). Small amounts of glycogen are often found in the interscapular fat body in relation to the normal nocturnal feeding cycle and larger amounts can be made to appear by forced feeding or by inducing alimentary hyperglycemia by refeeding after a period of fasting (Fawcett, '52). Many authors have suggested that this glycogen is metabolized locally in the adipose cells and that its appearance is causally related to the subsequent deposition of lipid (cf. Wertheimer and Shapiro, '48).

In addition to dietary factors several endocrine secretions affect the metabolism of adipose tissue. Insulin exerts a direct action on glycogen synthesis and lipid deposition both in vivo (Tepperman, Brobeck and Long, '43; Stetten and Boxer, '44; Tuerkischer and Wertheimer, '46; Renold, Marble and Fawcett, '50; Engel and Scott, '50) and in vitro (Wertheimer and Shapiro, '48; Haugaard and Marsh, '52). The lipid content of the interscapular brown fat body is increased under the influence of one or more pituitary hormones (Fawcett and Jones, '49; Baker, Ingle and Li, '50). On the basis of liver lipid analyses, Levin and Farber ('52) conclude that lipid mobilization from the fat depots requires at least two hormonal systems: the ACTH-adrenal cortical axis (cf. Ingle, '43) and a second, anterior pituitary factor, possibly the growth hormone.

It has been suggested that a testicular factor may be necessary for lipid mobilization in response to certain stimuli (Levin and Farber, '52). Adrenalin is also said to be involved in lipid mobilization (Clément, '51). However, these effects, like those of the thyroid (Fawcett and Jones, '49; Lachance and Pagé, '53) probably do not represent a direct action of these hormones on adipose tissue cells, but may affect fat depots secondarily by influencing other endocrine or nervous tissues.

The present study shows that the peripheral nervous system must be included in the list of factors modifying the metabolism of adipose tissue. The interscapular fat body

shows a transient deposition of glycogen and a decreased rate of lipid turnover in response to section of its nerve supply. Internal controls within each experimental animal, achieved by unilateral denervation, rule out effects secondary to the hyperglycemia which may follow damage to the nervous system (Bernard, 1858; Ogilvie, '52).

Just as the hormones interact with one another in antagonistic or synergistic fashion (Stadie, Haugaard and Marsh, '52) so must they interact with the nerve factor. For example, glycogen does not appear in brown fat of insulin-deficient (alloxan diabetic) rodents (Fawcett, '48) and presumably insulin deficiency would likewise limit the deposition of glycogen which we have described as a normal consequence of fat body denervation. On the other hand, the nerve may at times constitute the limiting factor, as during acute starvation at low temperature when lipid rapidly disappears from the intact fat body but is retained in the denervated fat body. These complex neuro-endocrine interrelationships can be resolved no further on the basis of presently available facts.

The altered metabolic properties of the denervated fat body may represent another example of Cannon's "law of denervation" which states that "when in a series of efferent neurons a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effects being maximal in the part directly denervated" (Cannon, '39; Cannon and Rosenblueth, '49). While this law makes no statement regarding the mechanism of the increased response, it does emphasize the common dependence of many types of end organs on their nerve supply. Tissues which show increased activity after denervation include smooth and skeletal muscle, sweat and salivary glands, adrenal medulla and several regions of the nervous system. Adipose tissue may now be added to this list, to the extent that increased glycogen and lipid deposition represents heightened sensitivity of denervated fat cells to circulating chemical substances.

Much of our experimental data is consistent with the hypothesis that the denervated fat body is more sensitive than the control to circulating insulin. Endogenous insulin, known to be essential for the deposition of glycogen in adipose tissue, may be a factor in the enhanced deposition of glycogen which follows denervation. Further, insulin stimulates the transformation of carbohydrate to lipid (Bloch, '52) as well as the incorporation of acetate into fatty acids (Haugaard and Stadie, '53) and may account at least in part for the increased concentration of lipid on the denervated side. The new methods used to elucidate pituitary-insulin antagonism (Stadie, Haugaard and Vaughn, '53) might be applied to test the hypothesis of nerve-insulin antagonism.

A finding not attributable to insulin on the basis of current knowledge is the defect in lipid mobilization from the denervated fat body. Another hypothesis (Hausberger, '37; Clément, '51) is that removal of an adrenergic neurohumor is responsible for this failure of lipid mobilization.

The property of the nerve responsible for the metabolic effects on adipose tissue is unknown. Because of the rapid onset of detectable changes (within 6 hours of denervation), one would be inclined to implicate nerve impulse conduction. It must be recalled, however, that nerves are capable of exerting profound influences on their end organs via a trophic effect unrelated to impulse conduction (Sidman and Singer, '51; Singer, '52). No evidence is available to indicate whether the nerve influence originates in the peripheral neuron or higher in the central nervous system. Finally, there is little evidence that the effect of nerve section is secondary to a vascular change. Nerve fibers in abundance appear to run independently of the blood vessels and to terminate directly on the adipose cells. Moreover no hyperemia has been noted grossly or microscopically in the denervated fat bodies. Inasmuch as the central origin of these nerve fibers has not been determined there is no reason to assume that they are sympathetic fibers (Hausberger, '39; Beznäk and Hasch, '37; Clément, '47).

SUMMARY

The normal innervation of brown adipose tissue in the mouse and rat has been described and illustrated and some metabolic consequences of interruption of these nerves have been studied by chemical and histological means. Numerous nerve fibers appear to terminate among the adipose cells while others accompany the major blood vessels. Under the stimulus of a 48-hour fast followed by refeeding, the denervated fat body deposits significantly more glycogen than does the innervated control in the same animal. In addition, a marked increase in lipid concentration is demonstrated at 4 and at 30 days postoperatively in the denervated fat bodies of mice maintained on a normal diet. During an acute fast at 5°C. the denervated fat cells retain lipid, whereas the controls become depleted. The effect is pronounced within 6 hours after denervation. The role of the nerve is discussed in relation to known effects of hormones on the metabolism of adipose tissue.

LITERATURE CITED

BAKER, B. L. 1952 A comparison of the histological changes induced by experimental hyperadrenocorticalism and inanition. Rec. Progress in Hormone Res., *7:* 331–373.

BAKER, B. L., D. S. INGLE AND C. H. LI 1950 Increase in glyceride content of brown fat by treatment with adrenocorticotropin. Proc. Soc. Exp. Biol. Med., *73:* 337–339.

BERNARD, C. 1858 Leçons sur la Physiologie et la Pathologie du Système Nerveux. J. B. Ballière, Paris.

BEZNÄK, A. B. L., AND Z. HASCH 1937 The effect of sympathectomy on the fatty deposit in connective tissue. Quart. J. Exp. Physiol., *27:* 1–15.

BLOCH, K. 1952 Interrelationships of lipid and carbohydrate metabolism. Ann. Rev. Biochem., *21:* 273–300.

BODIAN, D. 1937 The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. Anat. Rec., *69:* 153–162.

BOEKE, J. 1933 Innervationstudien. IV. Die efferente Gefässinnervation und der sympathische Plexus im Bindegewebe. Zschr. mikr. anat. Forsch., *33:* 276–328.

CANNON, W. B. 1939 A law of denervation. Am. J. Med. Sci., *198:* 737–750.

CANNON, W. B., AND A. ROSENBLUETH 1949 The Supersensitivity of Denervated Structures. Macmillan Co., New York, 245 pages.

CEDRANGOLO, F. 1937 Axione del sistema nervoso sul metabolismo dei glicidi e dei lipidi nel muscolo. Arch. di sc. biol., *23:* 377–398.

CLÉMENT, G. 1947 Demonstration directe de l'effet mobilisateur des hormones hypophysaires sur les graisses de réserve. Rôle du système nerveux sympathique. Compt. rend. soc. biol., *141:* 317–320.

———— 1951 Mobilization of reserve glycerides in the rat. III. Influence of hormonal factors. Arch. sci. physiol., *5:* 169–195.

CREASEY, N. H., AND C. H. GRAY 1952 Enzymes concerned in the synthesis of glycogen from glucose in the brown adipose tissue. Biochem. J., *50:* 74–81.

ENGEL, F. L., AND J. L. SCOTT, JR. 1950 The role of hormones in adipose glycogen synthesis in the rat. Insulin and the hyperglycemic factor of the pancreas. Endocrinology, *46:* 574–581.

FAWCETT, D. W. 1948 Histological observations on the relation of insulin to the deposition of glycogen in adipose tissue. Endocrinology, *42:* 454–467.

———— 1952 A comparison of the histological organization and cytochemical reactions of brown and white adipose tissues. J. Morph., *90:* 363–406.

FAWCETT, D. W., AND I. CHESTER JONES 1949 The effects of hypophysectomy, adrenalectomy and of thiouracil feeding on the cytology of brown adipose tissue. Endocrinology, *45:* 609–621.

HAUGAARD, E. S., AND W. S. STADIE 1953 The effect of hyperglycemic-glycogenolytic factor and epinephrine on fatty acid synthesis. J. Biol. Chem., *200:* 753–757.

HAUGAARD, N., AND J. MARSH 1952 Effect of insulin on the metabolism of adipose tissue from normal rats. J. Biol. Chem., *194:* 33–40.

HAUSBERGER, F. 1934 Über die Innervation der Fettorgans. Zschr. mikr.-anat. Forsch., *36:* 231–266.

———— 1935 Über die nervöse Regulation des Fettstoffwechsels. Klin. Wschr., *14:* 77–79.

———— 1937 Über die Veräunderung des Gehaltes an diastatischem Ferment im entnervten Fettgewebe. Z. ges. exptl. Med., *102:* 169–177.

———— 1939 Über Veränderungen des Fettansatzes im entnervten Fettgewebe. Arch. exp. Path. u. Pharmakol., *192:* 70–77.

HAUSBERGER, F., AND O. GUJOT 1937 Über die Veränderungen im Fett-, Wasser-, Glykogen- und Trockensubstanzgehalt im entnervten Fettgewebe. Arch. exp. Path. u. Pharmakol., *187:* 655–662.

ILLINGWORTH, B., AND J. A. RUSSELL 1951 The effects of growth hormone on glycogen in tissues of the rat. Endocrinology, *48:* 423–434.

INGLE, D. J. 1943 Relation of the adrenal cortex to the metabolism of fat. J. Clin. Endocrinology, *3:* 603–612.

KEN KURÉ, TOSHIO OI, AND SHIZEO OKINAKA 1937 Beziehungen des Spinalparasympatheticus zu der trophischen Innervation des Fettgewebes. Klin. Wschr., *16:* 1789–1793.

LACHANCE, J., AND E. PAGÉ 1953 Hormonal factors influencing fat deposition in the interscapular brown adipose tissue of the white rat. Endocrinology, *52:* 57–64.

LEVIN, L., AND R. K. FARBER 1952 Hormonal factors which regulate the mobilization of depot fat to the liver. Rec. Progress in Hormone Res., *7:* 399–435.

OGILVIE, R. F. 1952 Experimental Glycosuria: Its production, prevention, and alleviation. Vitamins and Hormones, *10:* 184–215.

RENOLD, A. E., A. MARBLE AND D. W. FAWCETT 1950 Action of insulin on deposition of glycogen and storage of fat in adipose tissue. Endocrinology, *46:* 55–56.

SCHOENHEIMER, R. 1942 The Dynamic State of Body Constituents. Harvard University Press, Cambridge, Mass., 78 pages.

SELYE, H., AND P. S. TIMIRAS 1949 Participation of "brown fat" tissue in the alarm reaction. Nature, *164:* 745–746.

SIDMAN, R. L., AND M. SINGER 1951 Stimulation of forelimb regeneration in the newt, *Triturus viridescens,* by a sensory nerve supply isolated from the central nervous system. Am. J. Physiol., *165:* 257–260.

SINGER, M. 1952 The influence of the nerve in regeneration of the amphibian extremity. Quart. Rev. Biol., *27:* 169–200.

STADIE, W. C., N. HAUGAARD AND J. B. MARSH 1952 The effect of growth hormone and cortisone on the action of bound insulin. J. Biol. Chem., *198:* 785–790.

STADIE, W. C., N. HAUGAARD AND M. VAUGHN 1953 The quantitative relation between insulin and its biological activity. J. Biol. Chem., *200:* 745–751.

STETTEN, D., JR., AND G. E. BOXER 1944 Studies in carbohydrate metabolism. III. Metabolic defects in alloxan diabetes. J. Biol. Chem., *156:* 271–278.

TEPPERMAN, J., J. R. BROBECK AND C. N. H. LONG 1943 The effects of hypothalamic hyperphagia and of alterations in feeding habits on the metabolism of the albino rat. Yale J. Biol. Med., *15:* 855–874.

TUERKISCHER, E., AND E. WERTHEIMER 1946 Factors influencing the deposition of glycogen in adipose tissue of the rat. J. Physiol., *104:* 361–365.

WERTHEIMER, E., AND B. SHAPIRO 1948 The physiology of adipose tissue. Physiol. Rev., *28:* 451–464.

PLATE 1

EXPLANATION OF FIGURES

1 Transverse section of spinal nerve at point of entry into brown fat body. Mouse. Bodian silver stain. × 550.

2 Interlobular bundle of nerve fibers. Rat. Bodian silver stain. × 550.

3 Interscapular brown fat body showing tissue architecture. Same fat body, same magnification as in figures 4, 5, 7, 9. Mouse. H and E. × 550.

4 and 5 Nerve fibers along interlobular blood vessel. Mouse. Bodian silver stain. × 550.

FFECT OF NERVES ON ADIPOSE TISSUE

RICHARD L. SIDMAN AND DON W. FAWCETT

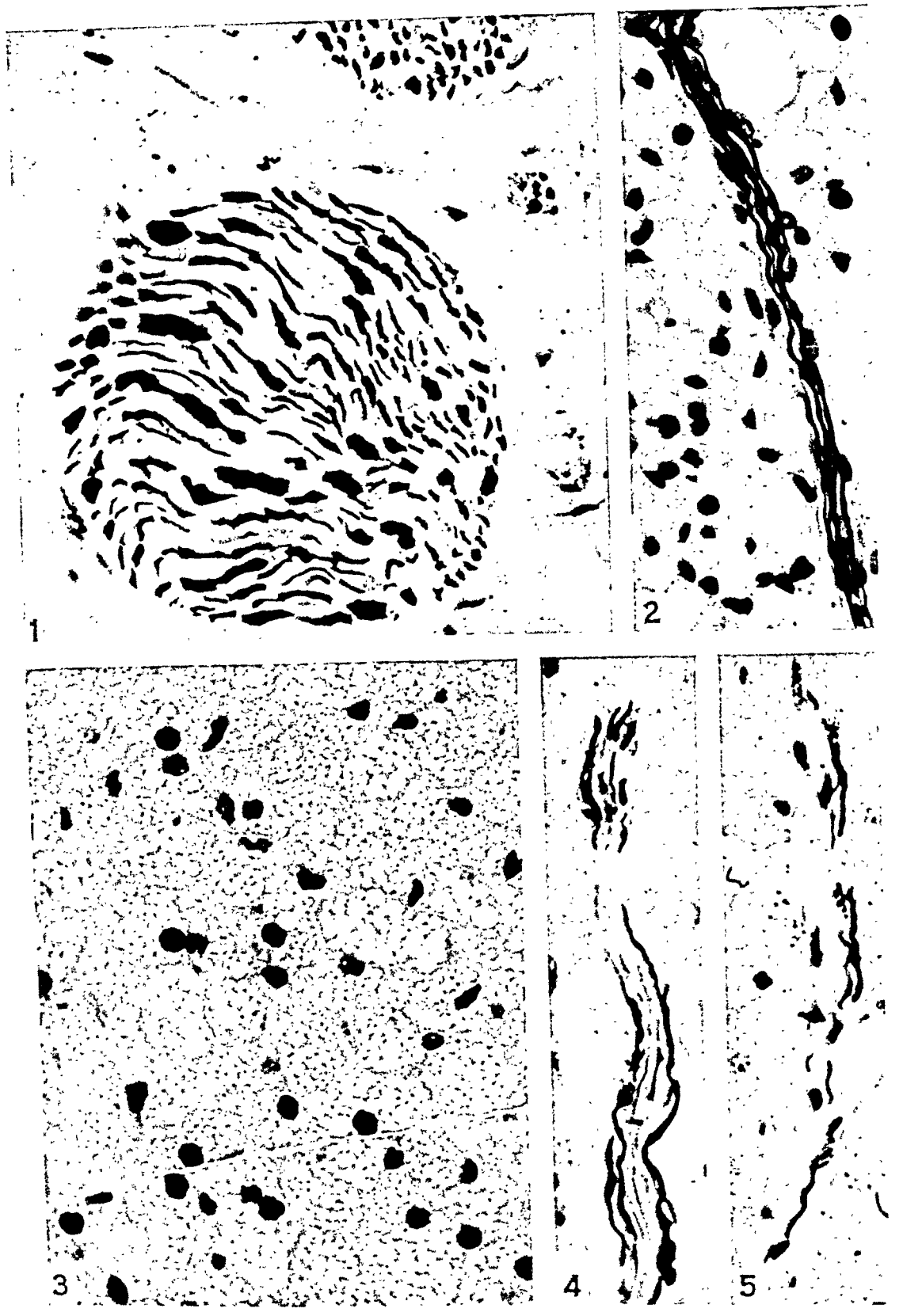


PLATE 2

EXPLANATION OF FIGURES

6 Small nerve in interlobular connective tissue branching and sending fibers into the adjacent fat lobule. Rat. Bodian silver stain. × 550.

7–10 Single nerve fibers among the cells of the interscapular fat. Figures 7 and 9 are from the mouse, 8 and 10 from the rat. Bodian silver stain. × 550.

EFFECT OF NERVES ON ADIPOSE TISSUE

RICHARD L. SIDMAN AND DON W. FAWCETT

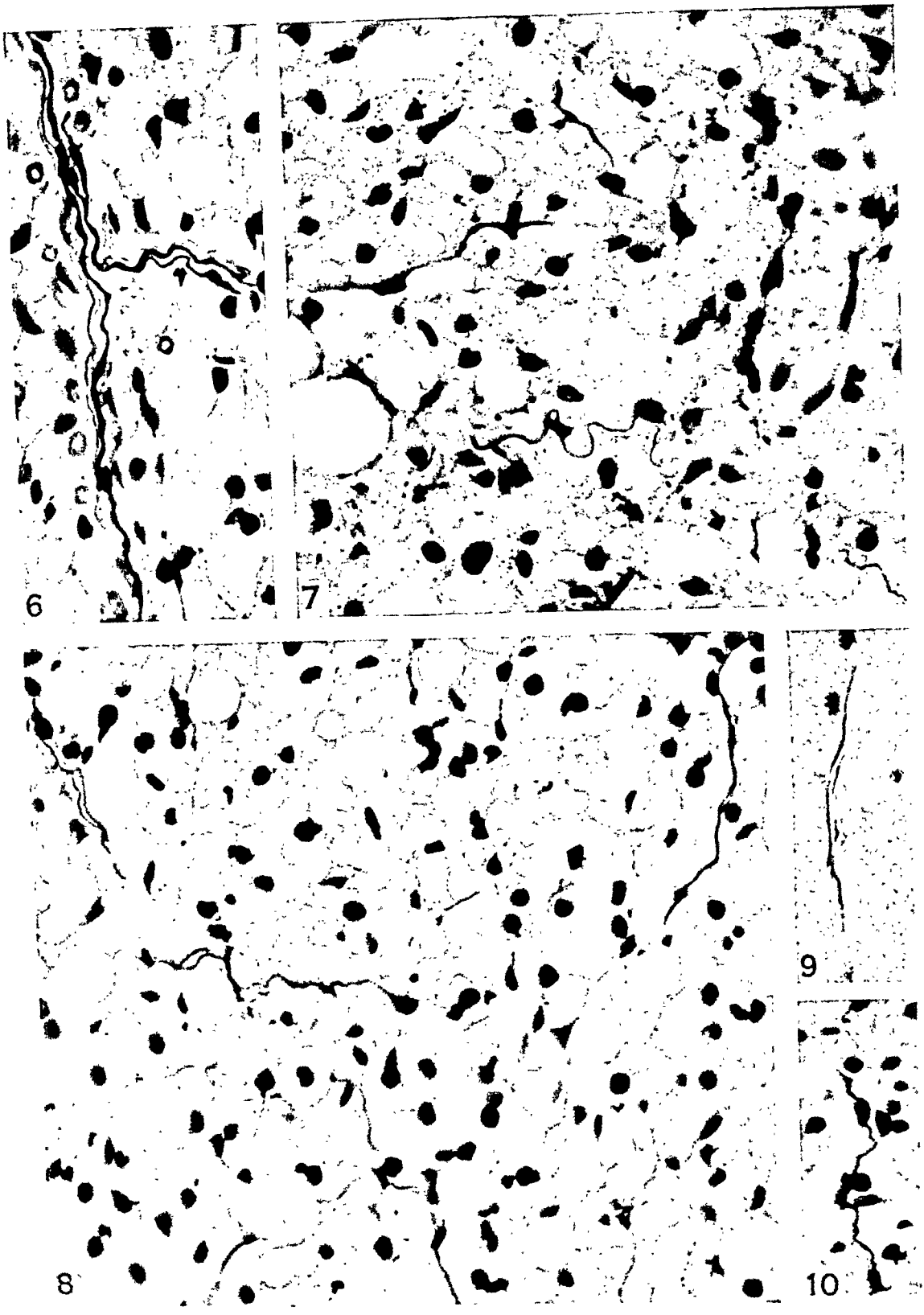


PLATE 3

EXPLANATION OF FIGURES

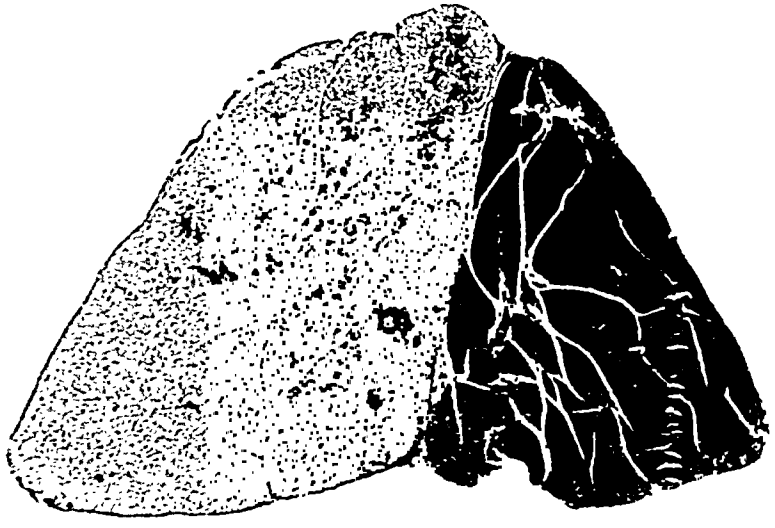
11 Fat bodies 4 days after denervation, diet *ad lib.* Denervated fat body on left shows abundance of lipid, which is represented by the empty spaces in this deparaffinized section; control fat body on right shows dark staining of cytoplasm and relatively little lipid. H and E. × 5.

12 Mouse fed *ad lib* for 24 hours after denervation of the fat body on the left. The denervated fat body contains abundant glycogen while the control (right) contains almost none. Periodic acid-Schiff stain. × 5.

13 Fat bodies of mouse treated similarly to that shown in figure 12. Considerable glycogen is present in the cytoplasm of the denervated fat cells on the left but not in the cells of the control side. Periodic acid-Schiff stain. × 350.

EFFECT OF NERVES ON ADIPOSE TISSUE

RICHARD L. SIDMAN AND DON W. FAWCETT



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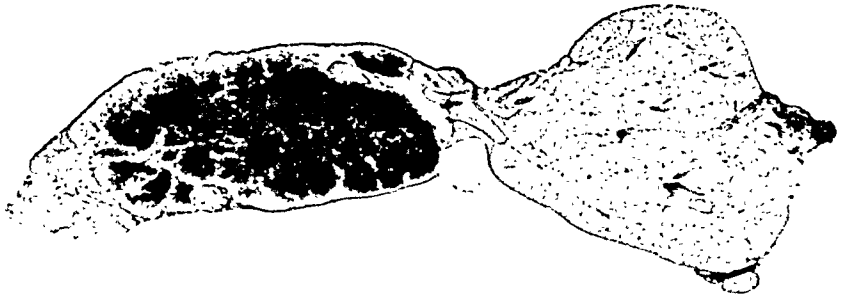

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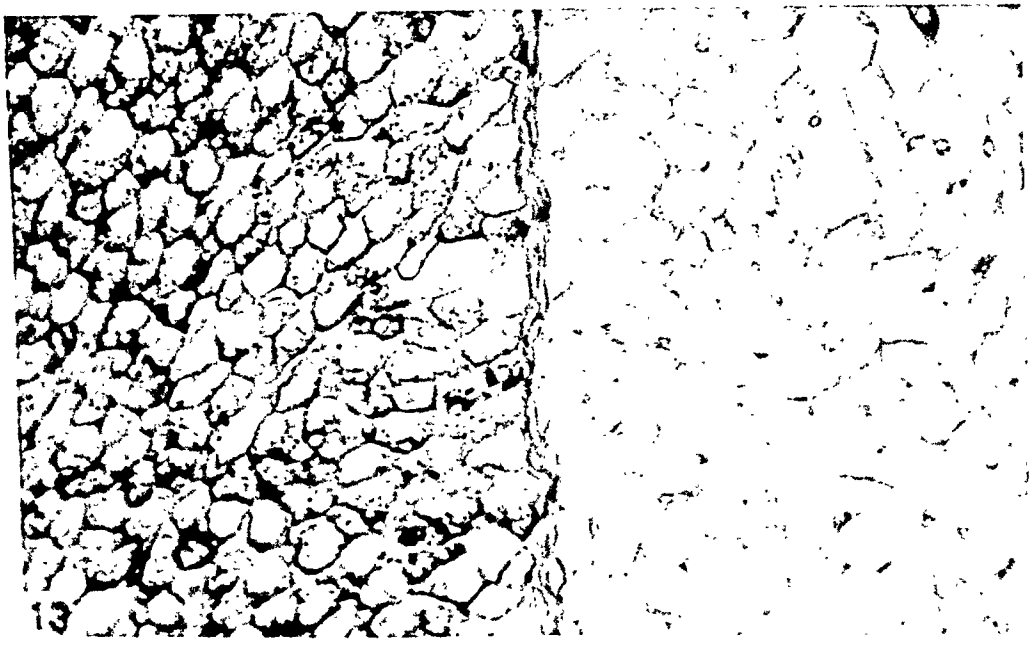

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PLATE 4

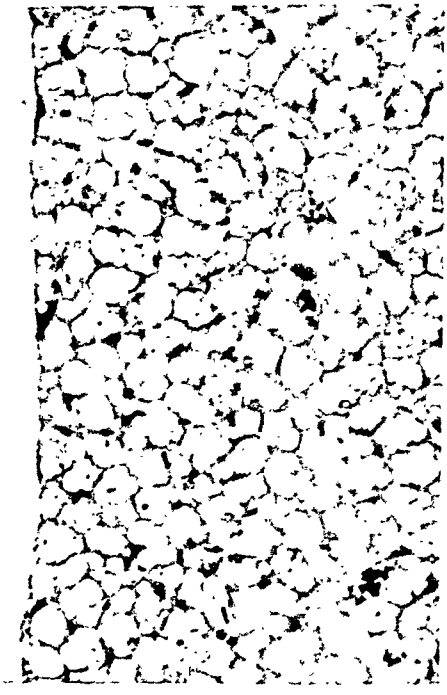
EXPLANATION OF FIGURES

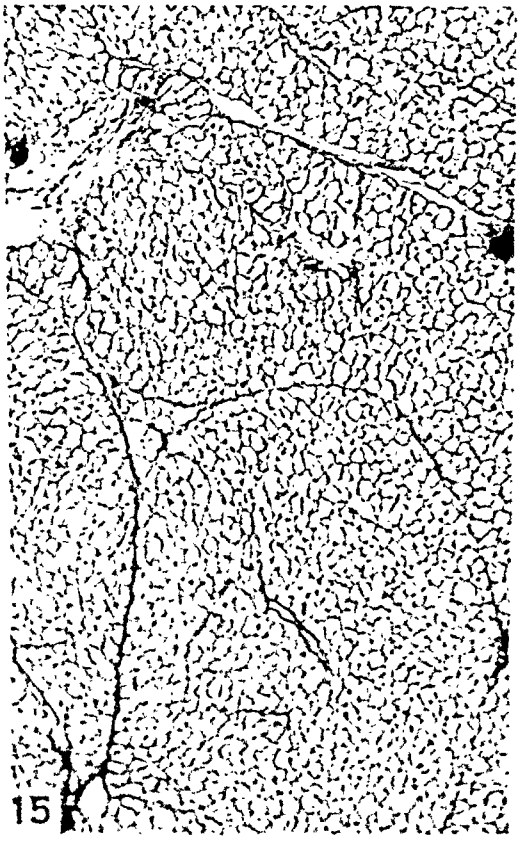
14 Fat bodies of mouse denervated unilaterally and maintained on a normal diet *ad lib* for 4 days postoperatively. The denervated fat body is on the right. H and E. × 220.

15 Normal control fat body of a mouse denervated unilaterally and maintained on normal diet *ad lib* for 30 days postoperatively. Periodic acid-Schiff stain. × 100.

16 The denervated fat body on the opposite side of the same mouse. Magnification is the same as in figure 15. Note increased size of lobules and of individual fat cells due to accumulation of lipid seen as empty spaces in this deparaffinized section. Periodic acid-Schiff stain. × 100.

14





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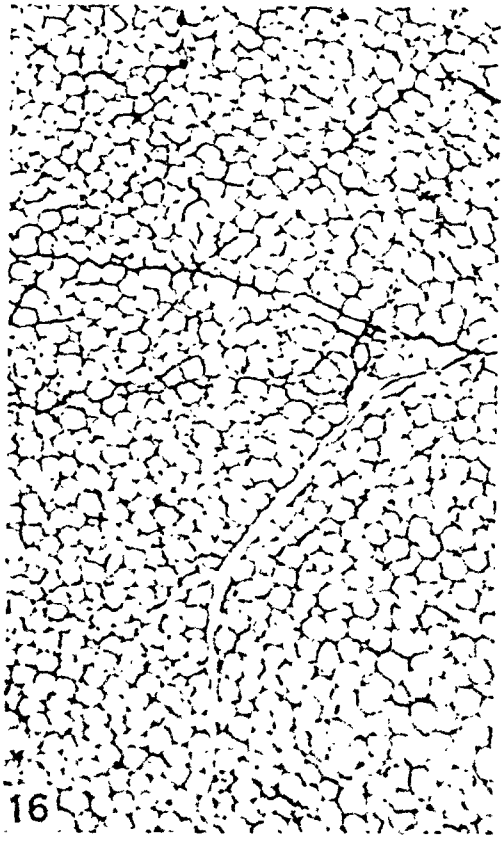

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PLATE 5

EXPLANATION OF FIGURES

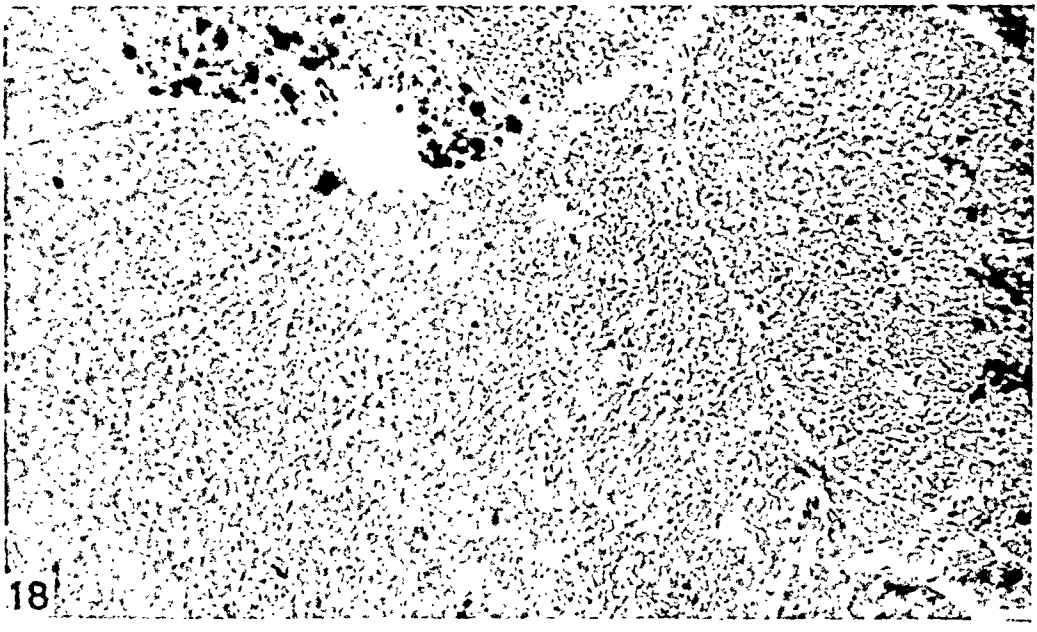
Brown fat bodies of a mouse denervated unilaterally, maintained postoperatively at 5°C. without food and sacrificed when moribund 6 hours later. All photographs represent frozen sections of formalin fixed fat bodies embedded in gelatin, sectioned at 10 μ and stained with sudan black B.

17 The denervated fat body is on the left and the innervated control fat body is on the right. Note retention of deeply stained neutral fat on the denervated side. Dark-staining tissue on the lower right is ordinary white adipose tissue. × 7.

18 Innervated fat body. Sudan stained neutral fat is lacking except in occasional groups of cells adjacent to blood vessels (upper left). × 100.

19 Denervated fat body. Large droplets of lipid are retained in nearly all of the cells. × 100.

17



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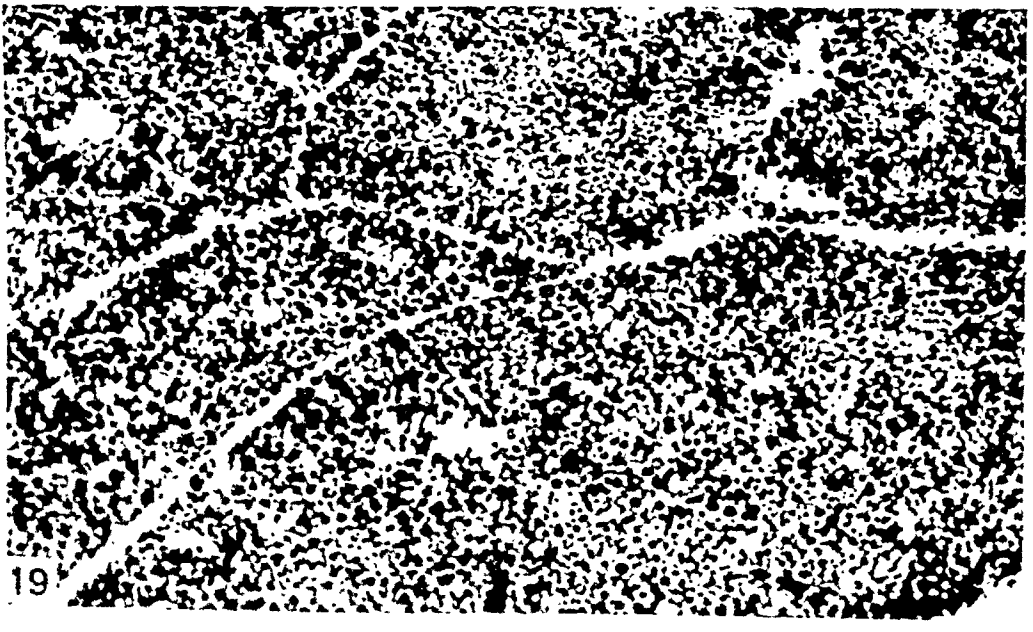

19

ESTERASES IN THE EARLY CHICK EMBRYO

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NINE FIGURES

The development of cholinesterase[2] activity in the embryogenesis of several animals has been studied by a number of previous investigators to obtain evidence for the importance of this enzyme in neuronal function. These studies were carried out to test the hypothesis that cholinesterase activity would increase significantly in the embryo at the time when nervous function began if this enzyme was important in synaptic transmission.

Youngstrom ('38) showed that cholinesterase was present in the embryos of three species of amphibia before the nervous system appeared and that the cholinesterase activity increased steadily from the beginning of motility to the early swimming stage. In *Rana spenocephalia,* it was found that cholinesterase was as abundant in the two and 4 cell dividing egg as it was in the early swimming stage embryo. Sawyer ('43a), studied the development of cholinesterase activity in whole embryos of *Amblystoma punctatum.* A close correlation between enzyme content and functional capacity was observed, though a small amount of the enzyme was detected in premotile em-

[1] Jeffries Wyman Scholar, Harvard University, 1952–1953. This work was supported in part by funds received from the Eugene Higgins Trust.

[2] The term "cholinesterase" used in papers published before 1943 refers to an enzyme which hydrolyzes acetylcholine and is inhibited by physostigmine in low concentrations. Thus, it may be either acetylcholinesterase of the nervous system or serum cholinesterase which is located in various tissues. Cholinesterase is synonymous with non-specific cholinesterase, "pseudocholinesterase," or type II cholinesterase. Acetylcholinesterase is synonymous with "true cholinesterase" or type I cholinesterase.

bryos. In a second study, Sawyer ('43b) observed that the cholinesterase detected in larval Amblystoma was primarily localized in nerve and muscle. As early as the neurula stage, cholinesterase could be detected in presumptive nerve and muscle. After peak enzyme activity had been reached in the feeding stage, a slow decline of activity occurred.

Ammon and Schütte ('34) studied the development of lipase, esterase, and cholinesterase in whole chick embryos, yolk sac, allantois and white and yellow yolk by chemical methods. Cholinesterase activity in individual embryos was not studied in embryos younger than 6 days of incubation.

In embryos of 6 or 9 days of age, Nachmansohn ('39b) determined the cholinesterase activity of chick nerve and muscle and found that cholinesterase levels rose steadily until about the 15th day and then more sharply until the time of hatching. Another study by Nachmansohn ('39a) traced the development of cholinesterase in embryonic muscle of several species including the chick.

Wenger ('51) studied the development of cholinesterase in several levels of the chick spinal cord in embryos of 5½ to 11 days' incubation.

A histochemical study of "lipase" in developing chick embryos has been made by Buño and Mariño ('52). These authors applied Gomori's method for "lipase" to a series of acetone fixed embryos. Hunter ('51) used the Gomori method to observe the distribution of esterase in 5 to 20 mm mouse embryos. Nachmansohn ('40) investigated cholinesterase activity in the spinal cord and brain of 60- to 136-day sheep embryos and Youngstrom ('41) studied the development of cholinesterase in the tissues of human fetuses. The earliest fetus in this study was 56 days of age.

Boell and Shen ('44) observed that cholinesterase activity was present in presumptive neural and skin ectoderm of *Amblystoma* but that it was not especially concentrated in the neural ectoderm until a definite neural plate had formed and neural folds had begun to close. Boell and Shen ('50) have

also studied the regional development of cholinesterase in the central nervous system of *Amblystoma*. The development of cholinesterase activity during the development of the grasshopper has been studied by Tahmisian ('43), and Augustinsson ('48) has studied this enzyme in the developing eggs of *Paracentrotus lividus*.

The majority of the studies cited above were concerned with the later stages of cholinesterase development in embryos which contained well established nervous systems. The studies of Sawyer ('43a, b), Youngstrom ('38), Boell and Shen ('44) and Tahmisian ('43) which include observations of embryos in the earliest stages of development, indicate that cholinesterase activity occurs very early in development at a time when the nervous system is not established. Even with the most delicate manometric procedures such as that used in the studies of Boell and Shen ('44), the exact localization of minute areas of enzymatic activity in very small embryos constitutes a major technical problem. Also, with the exception of Boell and Shen ('44, '50), these studies do not attempt to distinguish between the acetylcholinesterase of the nervous system and erythrocytes, and serum cholinesterase which is more widely distributed in animal tissues. Recently, histochemical methods for the demonstration of esterase (Nachlas and Seligman, '49a), serum cholinesterase (Ravin, Tsou and Seligman, '51) and acetylcholinesterase (Ravin, Zacks and Seligman, '53) have become available, so that an investigation of the more precise localization of these enzymes during embryogenesis now seems possible.

The present study consists of an examination of whole and serially sectioned chick embryos, of incubation ages 0 to 96 hours, by histochemical methods designed to reveal the presence of esterases in their specific sites of activity. Since the methods used are not entirely specific for the various enzymes in the esterase group, pretreatment inhibition experiments have been carried out in an attempt to characterize the enzymes which are present.

MATERIALS AND METHODS

Fertilized chicken (Plymouth Rock) eggs were obtained from a wholesale chicken farm and incubated at 103° for periods ranging from 0 to 96 hours. The embryos were staged by reference to Duval's atlas (1889). A group of unfertilized eggs was also examined.

Fresh whole embryo mounts

The embryos were removed from the egg, the vitelline membrane was separated from the embryo, and the albumen was carefully picked and washed away from the embryo in a dish containing 0.9% NaCl. It is quite difficult, but necessary to remove the albumen from the early stages since, if it is allowed to adhere to the blastoderm, it prevents access of the histochemical reagents. In later stages, the extra-embryonic membranes were also removed for the same reason. For whole embryo preparations, the embryo was floated into 25 ml of a solution containing Michaelis barbital buffer (pH 7.4), 0.9% NaCl, 6-brom-2-naphthyl acetate and diazo blue B where it remained for 20 minutes at 25–27°C. This supravital procedure was modified after the method of Ravin, Zacks and Seligman ('53).

After the reaction was complete, the reagent solution was pipetted off, and 10% neutral formol was pipetted onto the embryo. Especially in the early stages, extreme care must be exercised in manipulating the friable embryos since they are easily destroyed at this stage. After 10 minutes in 10% formol, the formol was pipetted off and the fixed embryo was washed with distilled water and floated onto a slide, drained and mounted in Kaiser's glycerogel. Preparations were examined immediately and drawings or photographs were made as required since the staining gradually deteriorated. Preparations stored for several weeks at 4°C. were still useful, but it should be emphasized that only fresh preparations are suitable for detailed study.

Serial sections of fresh frozen embryos

Early stages (0–36 hrs.) were prepared as whole embryo mounts as described above, but later stages (36–96 hrs.) were serially sectioned since the development of covering membranes and the increasing bulk of the embryo decreased the usefulness of the whole-embryo technic.

Embryos were removed from the egg as described for the whole-embryo preparations and were frozen immediately at —30°C. in a dish containing only the salt solution that adhered to the embryo. The frozen embryo was then imbedded in a 15% aqueous solution of gelatin and frozen rapidly at —30°C. Serial, 10 μ sections were cut in a Linderstrøm Lang cryostat as modified by Coons, Leduc and Kaplan ('51). The sections were dried on slides and then exposed to the histochemical reagents as described above. The sections were washed and mounted in glycerogel. Alternate serial sections were stained with hematoxylin to facilitate identification of the various structures.

In this histochemical procedure, the chromogenic substrate, 6-brom-2-naphthyl acetate is hydrolyzed by lipase, esterase and cholinesterases and the resulting 6-brom-2-naphthol then couples with diazo blue B which is present in the incubation solution. Sites of enzymatic activity are indicated by deposits of red-violet azo dye granules.

Since 6-brom-2-naphthyl acetate is hydrolyzed by lipase, esterase and the two types of cholinesterase, namely acetylcholinesterase and serum cholinesterase, it is necessary to use other substrates and inhibitors in an attempt to differentiate the different enzymes in this closely related family.

The chromogenic substrate, carbonaphthoxycholine iodide (Ravin, Tsou and Seligman, '51) was used as a specific indicator of serum cholinesterase activity in the manner described for 6-brom-2-naphthyl acetate. To test for the presence of this enzyme, whole embryos and sections were placed in solutions of carbonaphthoxycholine iodide and diazo blue B for 20 minutes at 25–27°C.

In pretreatment experiments designed to differentiate lipase and esterase from cholinesterase, sections were exposed to solutions of physostigmine (10^{-5} M.), and sodium taurocholate (10^{-3} M.) for 60 minutes. The solution was poured off into a flask and the histochemical reagents were added, mixed and then the mixture was filtered and returned to the sections for an additional 20 minutes. The reaction was allowed to proceed in the presence of the inhibitor in order to avoid reversal of inhibition.

Serial sections of fixed embryos

A series of embryos (24–96 hrs.) was fixed in cold acetone (4°C.) for 24 hrs., imbedded in soft paraffin (54–56°C.), cut at 10 μ and stained according to the method of Nachlas and Seligman ('49a).

Control experiments in which embryos were exposed to 10% formol for 10 min., or water at 90°C. for one minute prior to exposure to the histochemical reagents, never resulted in any azo dye deposition.

OBSERVATIONS

In fresh, unfertilized and unincubated blastoderms exposed for 20 minutes to 6-brom-2-naphthyl acetate and diazo blue B, no deposition of azo dye occurred, thus indicating absence of esterase activity. Portions of the vitelline membrane were diffusely colored violet, but typical granular deposits of dye were absent.

The earliest esterase activity was observed in a definitive streak stage of 15 hours' incubation. The anterior crescent of the blastoderm was filled with fine red-violet granules and a halo of coarser red-violet granules surrounded the area of the primitive pit and extended posteriorly along the primitive streak. The densest granular deposits were present in the region of Hensen's node. A few scattered granules were present in the area pellucida. Figure 1 illustrates the esterase activity of an embryo of this stage of development.

In the 21-hour embryo, the surfaces of the neural folds were covered with scattered red-violet granules of azo dye. The primitive streak region posteriorly was strongly reactive, the proamnion less so, and the areas pellucida and vitellina were slightly reactive. Few dye granules were observed in the area opaca. Figure 2 illustrates the sites of enzymatic activity in an embryo of this age.

By the 4-somite stage of development two major areas of esterase activity were observed in whole-embryo preparations.

Fig. 1 Diagram of a fresh, 15-hour definitive streak stage blastoderm, showing deposits of azo dye after 20 minutes in a mixture of 6-brom-2-naphthyl acetate and diazo blue B in a barbital buffer pH 7.4. Note the deposits of dye granules in the anterior crescent of the blastoderm and in the region of Hensen's node and the primitive streak. Traces of enzymatic activity are indicated by the few dye granules represented in the area vitellina interna.

The neural folds (fig. 3) showed prominent deposits of dye particles, which were most marked anteriorly along the closing edges. The sinus rhomboidalis was bisected by a dense, linear accumulation of dye granules which corresponded with

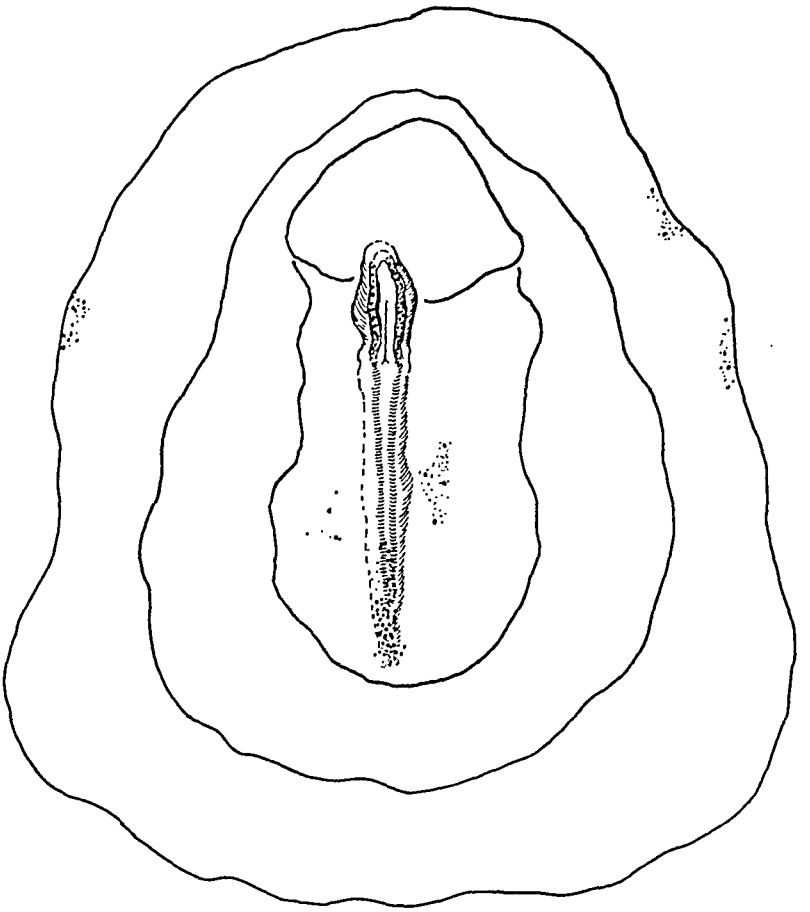

Fig. 2 Diagram of a 21-hour chick embryo after reaction with the histochemical reagents. Dye granule deposits are most prominent in the neural folds, and primitive streak region. Slight enzymatic activity is indicated by the scattered granules in the areas opaca and vitellina interna.

the location of the primitive streak. The anterior portion of the primitive streak contained a large concentration of dye granules. Dye granules were also diffusely scattered throughout the sinus region, but never as densely as in the central region. Few dye granules were observed in the areas pellucida or opaca, but the area vitellina interna contained somewhat

more dye. Figure 4 shows the distribution of enzymatic activity in a 4-somite embryo.

By 26 hours (8 somites), the surfaces of the prosencephalon and mesencephalon were covered with fine, punctate granules and the line of closure of the neural tube was marked by an especially intense dye deposition as was the edge of the

Fig. 3 Photograph of a portion of the still open neural folds in an embryo of 26 hours' incubation after reaction with the histochemical reagents. The dark spots represent deposits of azo dye which indicates the presence of esterolytic activity. × 440.

neural folds more posteriorly in the region where closure was incomplete. Considerable activity was present in the central portion of the sinus rhomboidalis in a pattern similar to the 24-hour stage. Dye granules were also present in the intersomitic furrows and on the dorsal surface of the somites. The greatest concentration of granules on the somites was located in the medial portion of the somites adjacent to the neural tube (fig. 5). The amniotic fold contained red violet granules and the area pellucida scattered dye granules, whereas the

area vasculosa was primarily unreactive. Figure 6 illustrates the appearance of the 26-hour embryo.

In the 34-hour (13 somites) embryo, the esterase activity of the neural folds, sinus rhomboidalis, amnion and a zone on either side of the embryo was marked. Some granules

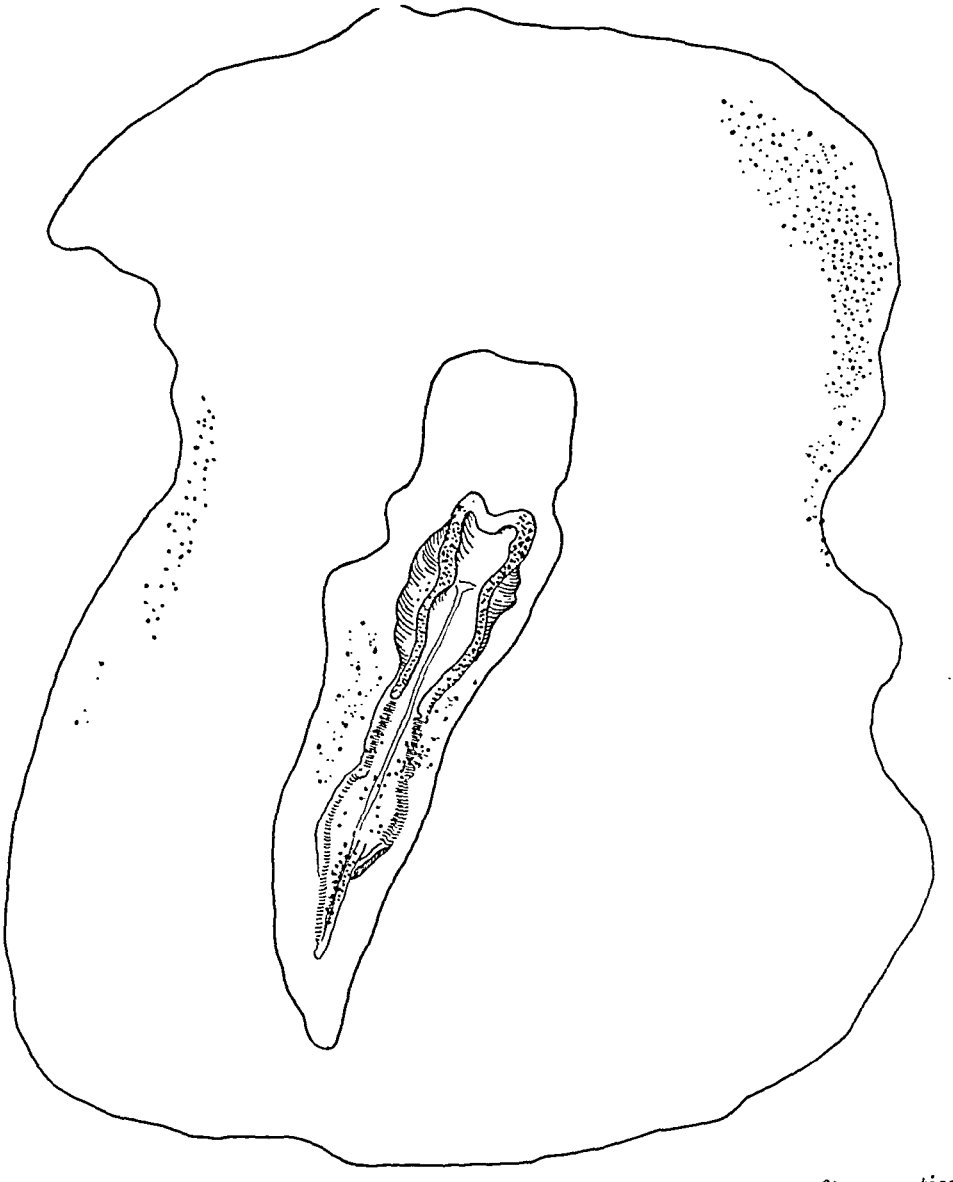

Fig. 4 Diagram of a 4-somite (23-hr.) whole-embryo preparation after reaction with the histochemical reagents. The neural folds, somites and primitive streak are most reactive. More enzymatic activity is seen in the area opaca in this stage than in the 21-hour embryo.

were present in the area vasculosa and vitellina interna, but much less than that seen in the areas immediately lateral to the embryo. As in 24- and 26-hour stages, the primitive streak in the sinus rhomboidalis area was marked by a dense line of dye granules (fig. 7). Somites were covered with dye granules as in the 26-hour stage.

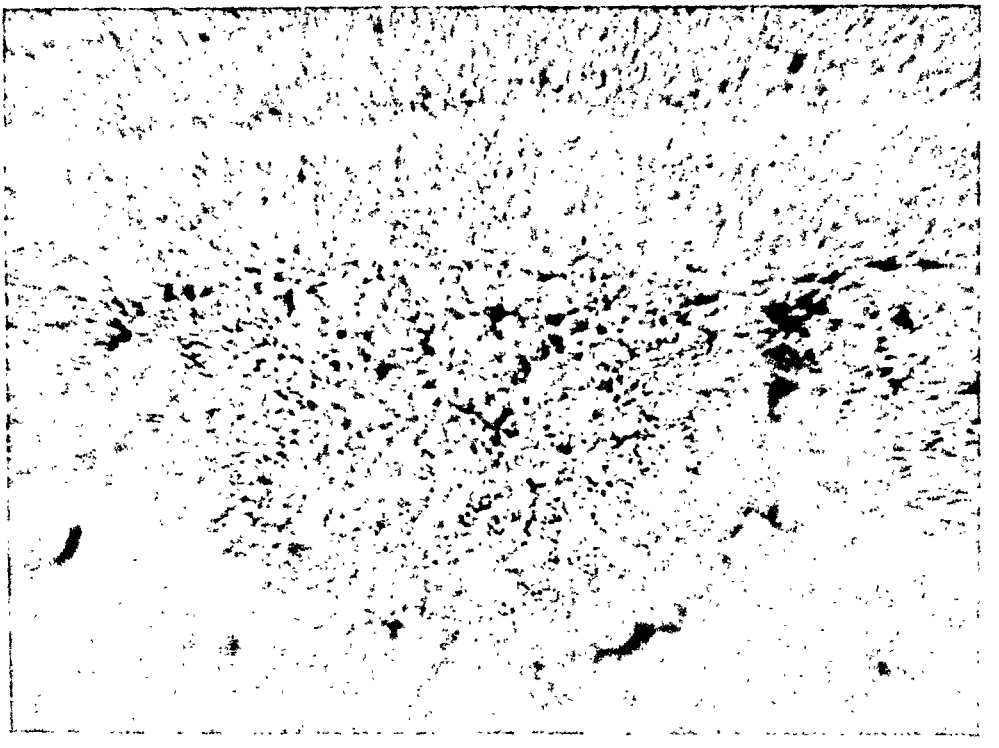

Fig. 5 Photograph of the neural fold and somites of an 8-somite chick embryo after reaction with the histochemical reagents. The surfaces of the neural tube and the somite are covered with azo dye granules. × 440.

In whole mount preparations of stages of 34 hours or older, less dye deposition occurred over the region of the closed neural folds, probably due to the interference of the somatic ectoderm which prevented access of the histochemical reagents to the underlying neural tube. To avoid this difficulty, later stages were sectioned serially as described in the section on methods. Figure 8 shows esterase activity in a 13-somite embryo.

In sectioned 48- to 52-hour chick embryos, little esterase activity could be demonstrated in transverse sections of the brain. Only an occasional dye granule could be seen in the developing brain and anterior neural tube, but slightly more activity was present in the posterior neural tube. These granules were scattered over the transverse section of the

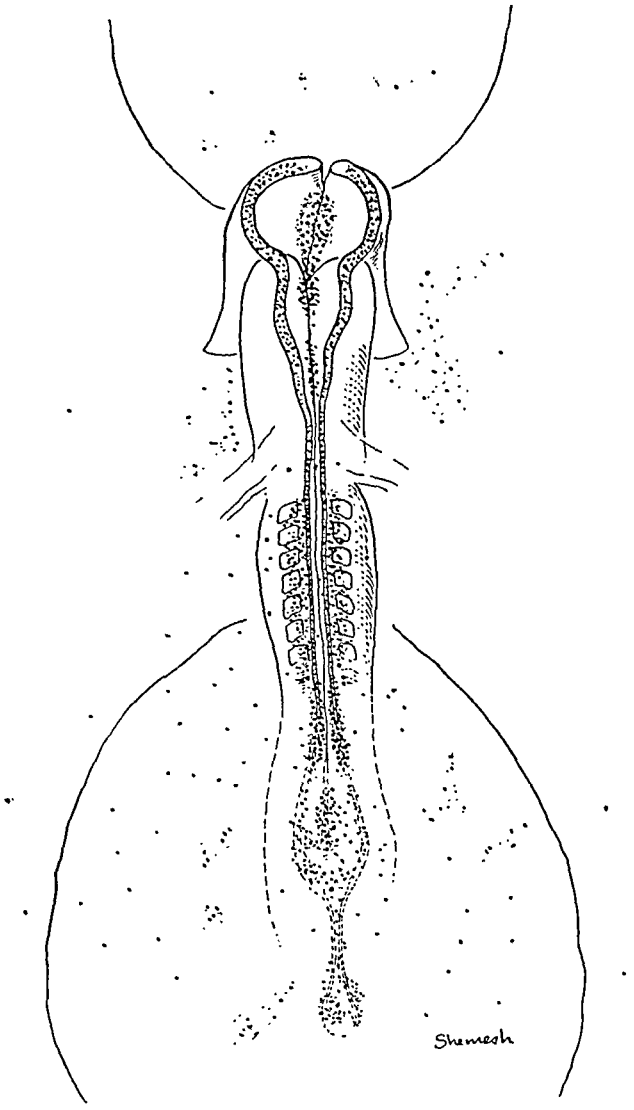

Fig. 6 Diagram of a 26-hour (8-somite) embryo after reaction with the histochemical reagents. Azo dye granules are present on the brain, neural folds, somites and in the primitive streak region. Increased enzymatic activity is present in the extra-embryonic areas.

neural tube but it was not possible to determine which cells contained esterase activity. At this stage of development the spinal cord contains ependymal and germinal cells, but lacks neuroblasts which do not appear until the third day (Lillie, '29). The areas vasculosa and opaca contained dye

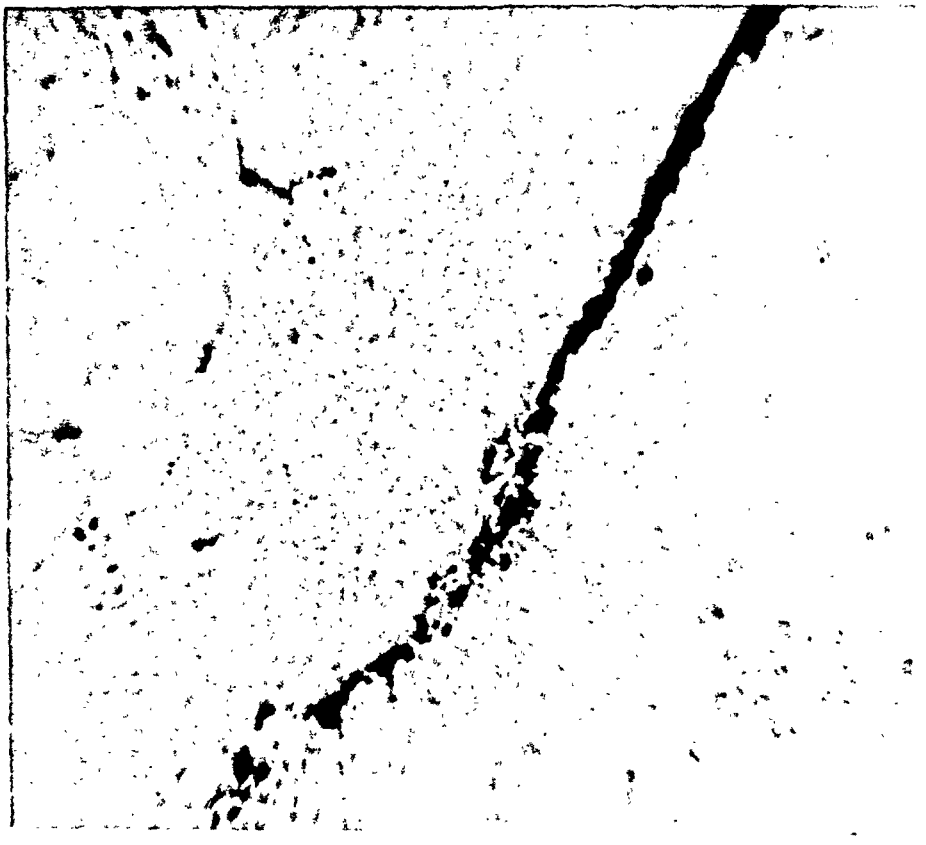

Fig. 7 Photograph of the primitive streak region in an 8-somite chick embryo whole mount after reaction with the histochemical reagents. Note the intense deposit of azo dye particles bisecting the area rhomboidalis. × 440.

granules. The most intense dye deposition occurred in the yolk sac endoderm where coarse blue-violet dye granules intimately surrounded the yolk globules.

The chick embryos of 68 to 72 hours of incubation contained dye granules in the amnion, foregut, notochord and areas vasculosa and vitellina interna. The dye deposition around the yolk globules (fig. 9) was particularly intense and the

yolk sac endoderm contained many fine and coarse dye granules. Very little dye deposition was observed in the brain or anterior neural tube, although some dye granules were observed in the posterior neural tube similar to those seen in the 48- to 52-hour embryos.

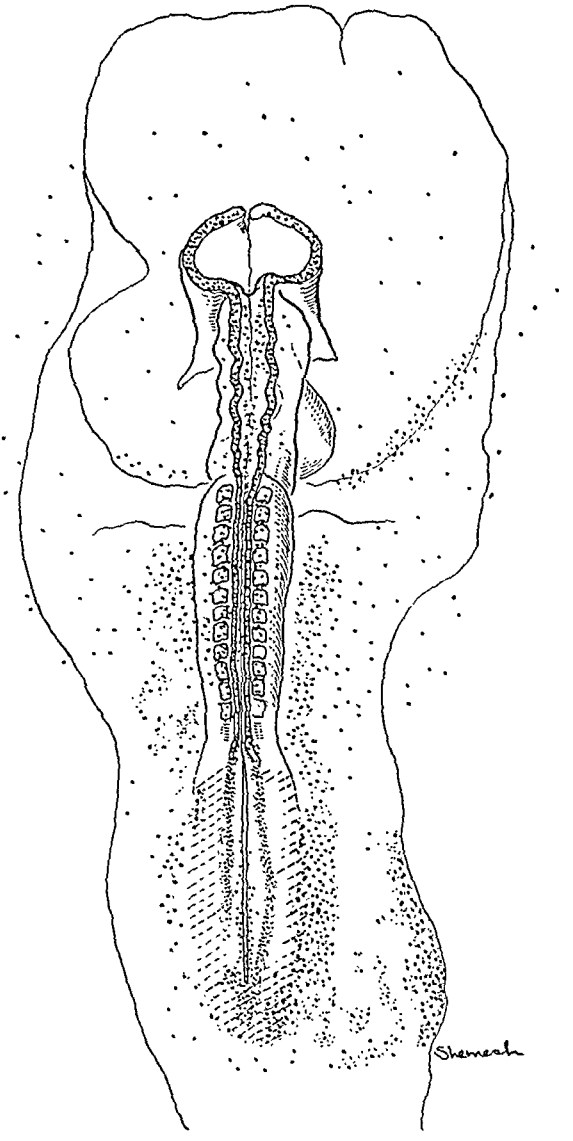

Fig. 8 Diagram of a 13-somite (34-hour) embryo after 20 minutes in the histochemical reagents, 6-brom-2-naphthyl acetate and diazo blue B. Note the deposits of azo dye in the brain, neural tube, somites and primitive streak area. Considerable dye deposition is also present in the extra-embryonic area.

In the 96-hour embryo, the brain contained a few scattered dye granules and small clusters of granules in the metencephalon (possibly the nucleus of V). The retina and lens were filled with red-violet granules. Increased reactivity was also noted in the posterior neural tube. The amnion (outer margin especially), fore- and hindgut, yolk sac endoderm, heart and notochord were reactive. The densest accumulations of dye were present in the yolk sac endoderm, amnion and notochord. Table I semiquantitatively summarizes the results of the observations.

Inhibition experiments

As discussed in the section on methods, the substrate 6-brom-2-naphthyl acetate is hydrolyzed by lipase, esterase, and

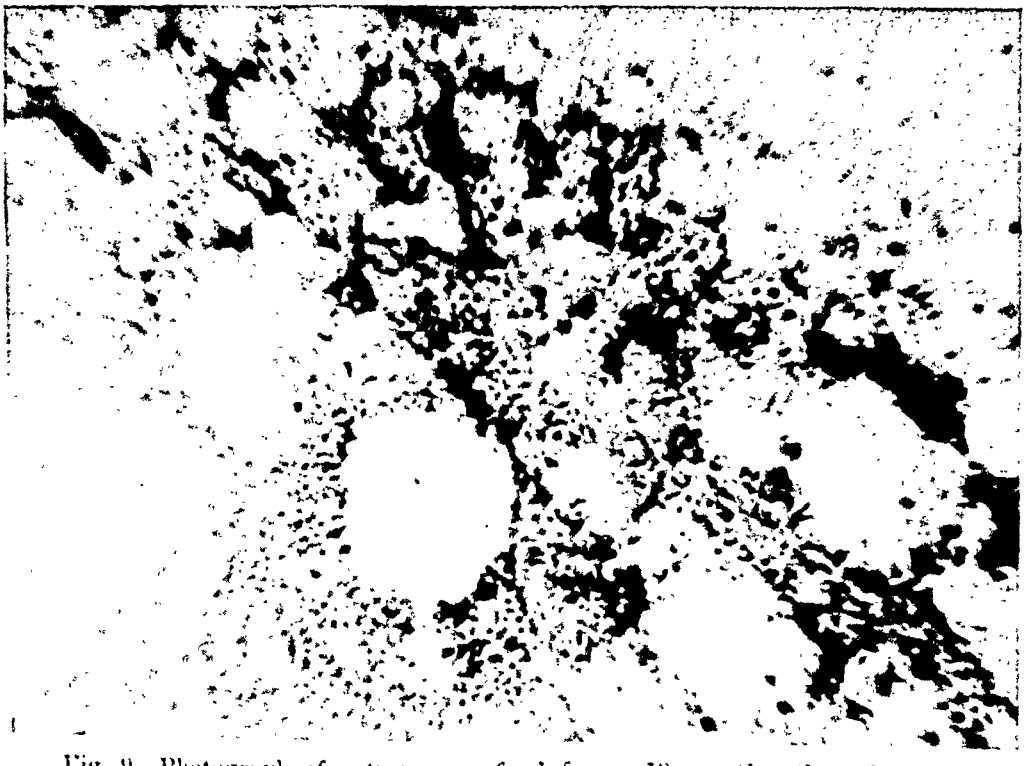

Fig. 9 Photograph of a transverse fresh-frozen 10 μ section through the yolk sac endoderm of a 96-hour chick embryo after reaction with the histochemical reagents. The clear areas represent yolk globules which are surrounded by dense deposits of azo dye.

the two major types of cholinesterase, namely acetylcholinesterase and serum cholinesterase. Since cold acetone fixation destroys cholinesterase activity, any surviving esterolytic activity demonstrable with 6-brom-2-naphthyl acetate is due to

TABLE 1

Esterases in the developing chick embryo

REACTIVE AREAS	HOURS OF INCUBATION								
	0	15	21	24	26	36	48–52	68–72	96
Germinal disk	. . .	....	....	....	..	....	....	....	....
Ant. crescent	. .	+	....	....	...	....	....	....	....
Hensen's node		+	....	....	..	....	....	....	....
Prim. streak		.	+	++	++	++	....	....	....
Neural folds	..	..	+	++	+++	++	....	....	....
Proamnion	.	. .	+	+	+	+	....	....	....
Area pellucida	.	±	+	+	+	+	....	....	....
Area opaca	. . .	. .	+	+	..	....	....	....	....
Area vasculosa	....	....	....	....	..	+	+	+	....
Area vitellina	....	....	....	+	..	+	++	....	....
Sinus rhomboidalis	...	....	....	+	+	+	....	....	....
Prosencephalon	....	....	....	....	+	....	....	....	....
Mesencephalon	.	....	....	....	+	....	....	....	....
Metencephalon	....	....	....	....	...	....	....	....	+
Intersomitic furrows	....	....	....	....	+	+	....	....	....
Somites	....	....	....	....	+	+	+	....	....
Amnion	. .	....	....	....	+	+	+	+	++
Yolk sac endoderm	.	....	....	....	..	....	+	++	++
Foregut	...	....	....	....	..	....	....	+	+
Hindgut	..	....	....	....	...	....	....	....	+
Ant. neural tube	....	....	....	....	..	....	±	±	....
Post. neural tube	....	....	....	....	..	....	+	+	+
Retina	...	....	....	....	..	....	....	....	+
Lens	....	....	....	....	...	....	....	....	+
Heart	....	....	....	....	....	....	....	±	+
Notochord	...	....	....	....	...	....	....	+	++

either lipase or aliesterase. Lipase can be differentiated from esterase by the selective inhibition of the latter enzyme by sodium taurocholate (Nachlas and Seligman, '49b). Lipase is either activated or unaffected by 10^{-3} M., sodium taurocholate. Cholinesterase activity is selectively inhibited by low concentrations of physostigmine (Easson and Stedman, '37;

Richter and Croft, '42). The two major types of cholinesterase can be differentiated histochemically by the use of cold acetone, physostigmine and the specific serum cholinesterase substrate, carbonaphthoxycholine iodide. An acetone and physostigmine-sensitive enzyme which hydrolyzes 6-brom-2-naphthyl acetate, but not carbonaphthoxycholine iodide, will be regarded as acetylcholinesterase in the following discussion.

After cold acetone fixation of a series of embryos (30–96 hrs.) and imbedding and staining according to the method of Nachlas and Seligman ('49a), only the area opaca and yolk sac endoderm of the older stages (48–96 hrs.) contained dye granules. Thus it appears that two types of esterolytic enzymes are present in the chick embryo; the enzyme of the embryonic axis which is inhibited by acetone fixation, and the enzyme associated with the yolk sac endoderm which is much less sensitive to acetone.

Inhibition experiments with physostigmine alkaloid (10^{-5} M.), showed that all staining in the embryonic axis was abolished and slightly reduced in the extra-embryonic membranes when the substrate and coupling reagent were subsequently added. Inhibition by this concentration of physostigmine may be cited as evidence for the presence of cholinesterase activity. The specific chromogenic substrate for serum cholinesterase, carbonaphthoxycholine iodide, was not hydrolyzed either by the enzyme of the embryonic axis or the enzyme in the extra-embryonic membranes. The absence of azo dye formation when this substrate was used is evidence for the absence of serum cholinesterase. Thus, the enzymes responsible for 6-brom-2-naphthyl acetate hydrolysis appear not to be of the serum cholinesterase type.

Inhibition experiments utilizing sodium taurocholate (10^{-3} M.), produced equivocal results. Activation was never observed, but inhibition of the extra-embryonic membranes variably occurred.

Until more specific histochemical reagents are prepared or other methods are available for analysis of minute fragments of tissue, the exact identification of the enzymes, as demon-

strated by kinetic and other biochemical criteria, must be deferred. On the basis of the inhibition procedures, it appears that the embryonic axis contains acetylcholinesterase and the extra-embryonic membrane, lipase, or more probably aliesterase.

DISCUSSION

Two types of esterase can be demonstrated by histochemical means in the early chick embryo. The enzyme of the embryonic axis is inhibited by low concentrations of physostigmine and by cold acetone. This enzyme first appears in the definitive streak stage of 15 hours' incubation and its activity is localized in the anterior crescent of the blastoderm, Hensen's node, and in the primitive streak. As development proceeds, the acetylcholinesterase activity is chiefly localized on the surface of the neural folds, especially along the closing edges, and in the primitive streak area up to the 34-hour stage. As the embryo continues to develop, enzymatic activity appears on the surface of the prosencephalon (26 hrs.) and in the region of the somites in whole embryo preparations of 34 hours' incubation. The activity of the primitive streak begins at 15 hours and is prominent in the sinus rhomboidalis of older embryos (24 to 36 hrs.).

In serially sectioned older stages (30 to 96 hrs.), it is notable that extremely little enzymatic activity could be demonstrated in the brain and anterior portions of the neural tube, except in certain specific locations such as the retina and circumscribed spots in the metencephalon. The posterior neural tube contained some enzymatic activity in sections of 48- to 96-hour embryos. In earlier stages (20 to 26 hrs.) examined by the whole mount technique, the enzymatic activity was most prominent in the neural folds, while in older, sectioned embryos little activity could be demonstrated. This may be due to the disappearance of the enzyme in the older embryos or a dilution effect caused by an increase of brain mass without a concomitant increase in acetylcholinesterase content. Sawyer ('43b) attributed the decline in nervous system cholinesterase in 60-day *Amblystoma* embryos to the dilution of

enzyme-rich components by the disproportionate increase of enzyme-poor components.

Support for the idea that the enzyme concentration may have decreased might be derived by analogy from the observations of Levy and Palmer ('40, '43) who showed that dipeptidase and amino peptidase activity decreases from their initial levels during the first few days of development. Moog's ('43a, '44) studies on acid and alkaline phosphatase activity in chick embryos indicate that high concentrations of these enzymes occur diffusely distributed during the early stages of development and later decrease when they become more specifically localized.

Moog ('44, '52) has also suggested that changes in enzymatic activity during embryonic development may in part be due to changes in the characteristics of enzyme molecules already present.

Several studies by Youngstrom ('40), Nachmansohn ('39, '40), Wenger ('51), Sawyer ('43a, '43b) and others have established that cholinesterase levels in the nervous system increase markedly when functional activity begins. This has been taken as evidence in support of the role of acetylcholine and cholinesterase in neuromuscular transmission. In noting these facts, however, it should not be overlooked that cholinesterase appears before synaptic function is possible.

The studies of Youngstrom ('38), Sawyer ('43a, '43b) and Boell and Shen ('44) indicate that cholinesterase is present in early embryos before neuronal differentiation has proceeded to a stage where function is possible. Sawyer ('43b) demonstrated cholinesterase activity in the presumptive nerve and muscle of premotile *Amblystoma* embryos (stages 12 to 14), although Boell and Shen ('44) were able to demonstrate cholinesterase activity in their preparations only after a definite neural plate had formed and the neural folds had begun to close.

The histochemical method employed in this study demonstrates a physostigmine-sensitive enzyme (acetylcholinesterase) in Hensen's node and the primitive streak of 15-hour

chick embryos, which is the earliest appearance of esterase activity in this species.

It is of interest that Hensen's node and the primitive streak should show early and intense acetylcholinesterase activity. The node region gives rise to the head and nervous system according to the experiments of Rawles ('36), Rudnick ('44, '48) and Spratt ('52).

The node in the early embryo is also the site of intense cytochrome oxidase activity (Moog, '43). Spratt ('51) has shown that the areas of most intense reducing activity, as demonstrated by tri-phenyl or neotetrazolium chloride, are the node and head fold in head process blastoderms; and the node, segmental plate, posterior end of the primitive streak and forebrain in early somite blastoderms. This pattern of reducing activity is quite similar to the distribution of esterolytic activity in the early chick embryo demonstrated in this study. Also, node and forebrain show the greatest degree of cytoplasmic basophilia (Gallera, '48).

It is also of interest that the node stains differentially with Janus green B (Rulon, '35). This may be due to the presence of cytochrome oxidase as suggested by Lazarow and Cooperstein ('53) and to the presence of cholinesterase. The parallel occurrence of cholinesterase activity and Janus green B staining has been observed in motor end plates (Welsh and Zacks, '49), rat liver mitochondria (Zacks and Welsh, '51), and the blood cells of certain invertebrates (Zacks, '53). Rulon's ('35) observation that developing neural folds are selectively stained by Janus green B and methylene blue is also of interest in this connection.

The possible role of acetylcholinesterase activity in the nervous system of the early embryo is not clear. Boell and Shen ('44) regard the presence of cholinesterase as evidence of localized action of its specific substrate (Boell and Nachmansohn, '40) and Sawyer ('43a) believes that *adequate levels* of the enzyme must be present before function is possible. This must be the case in the chick, since neuroblasts do not appear until the end of the third day of incubation

(Lillie, '29) and reflex activity does not begin until the 4th or 5th day of incubation (Windle and Orr, '34; and Kuo, '32, '38). The observations of Kuo ('39) indicate that acetylcholine, the specific substrate of acetylcholinesterase, does not appear in the nervous system of chick embryos until two and one-half days of incubation. Furthermore, this author states, "it is highly probable that if sufficient embryos, younger than two and one-half days had been available, it would have been possible to detect acetylcholine in the embryo of two days or even younger." The work of Szepsenwol and Caretti ('42) indicates that acetylcholine first appears in the spinal cord of the chick embryo on the second day of incubation. Subsequent to the 6th day, the acetylcholine level falls but rises once again on the 20th day of incubation. Acetylcholine appears in the spinal cord before the level in the brain begins to rise.

In developing grasshoppers, Tahmisian ('43) observed that during the prediapause, from the day of laying until the 7th day of development, no cholinesterase can be detected in the developing eggs. On the 7th day, cholinesterase activity becomes detectable although no acetylcholine can be demonstrated until the 17th day postdiapause. Unlike the chick embryo, which lacks both acetylcholine and neuroblasts at a time when cholinesterase activity can be detected, the grasshopper embryo shows a correlation between the appearance of cholinesterase and the development of neuroblasts which appear on the 7th to 9th day of the prediapause.

Thus, in several species, cholinesterase activity can be detected during embryonic development before functional activity of the nervous system is possible. In the chick, acetylcholinesterase can be detected in the presumptive and developing nervous system before neuroblasts and synapses appear. Also, acetylcholinesterase activity can be detected before the substrate, acetylcholine, appears in the embryo. Apparently, a prelude of enzyme activity precedes the completion of the enzyme-substrate system as the embryo continues to develop. It is of considerable interest that the enzyme activity precedes the appearance of the specific substrate. A

similar situation was observed in the developing grasshopper by Bodine ('41) who showed that tyrosinase activity can be detected in the developing grasshopper before melanin formation occurs.

The significance of the early appearance of acetylcholinesterase in the embryo is unknown. Unlike enzymes known to be vital in cellular metabolism such as dehydrogenases, oxidases and phosphatases, the current view of cholinesterase places it in a group of more highly specialized enzymes designed for special functions (Moog, '52). However, the finding of acetylcholinesterase in erythrocytes (Alles and Hawes, '42; Augustinsson, '48) suggests that acetylcholinesterase may have functions other than trans-synaptic transmission and possibly axonal conduction. It should be emphasized that in the early studies (prior to 1943) of cholinesterase development during embryogenesis, no attempt was made to differentiate acetylcholinesterase from serum cholinesterase. Boell and Shen ('44) showed that mecholyl hydrolyzing capacity of stage 16 *Amblystoma* embryos was approximately equal in both nervous system and ectoderm. The ability to hydrolyze mecholyl is regarded by Mendel and associates ('43) as indicative of the presence of acetylcholinesterase. Esterase and serum cholinesterase do not attack this substrate according to these authors. In the present study, the failure of early chick embryo nervous system to hydrolyze the chromogenic substrate, carbonaphthoxycholine iodide is taken as evidence of the absence of serum cholinesterase. Thus, only in the embryonic nervous system of *Amblystoma* and the chick is there evidence for the early occurrence of acetylcholinesterase.

In the discussion of the development of cholinesterase activity in the embryogenesis of the grasshopper, Tahmisian ('43) speculates that this enzyme may have an inductive role. This author cites the observations of Youngstrom ('38) who found that cholinesterase was present before the induction of the nervous system in several amphibians.

Boell and Shen ('44) showed that cholinesterase appeared in induced neural tissue when chorda material was implanted. The presence of cholinesterase activity in the transplanted material of stage 10 was not determined, but apparently was absent, since the first detectable enzymatic activity was reported in embryos of stages 12 to 14. The present observations on the development of acetylcholinesterase in the chick embryo provide little data to support the hypothesis of an inductor role of this enzyme in embryogenesis. Acetylcholinesterase was found in the presumptive neural plate and later in the neural folds. By 68 hours, the notochord was strongly reactive. In the 96-hour embryo, the retina and lens contained acetylcholinesterase. Only the differentiating lens ectoderm over the optic vesicle showed acetylcholinesterase activity as contrasted with somatic ectoderm elsewhere. This might suggest that the cholinesterase-containing optic vesicle and differentiating retina were operating to induce cholinesterase activity in the overlying lens ectoderm. Lindeman ('47) studied the development of cholinesterase and acetylcholine in the chick retina in an attempt to correlate the appearance of these substances with function. However, since cholinesterase activity was measured from the 8th day of incubation, no information was acquired concerning the early stages of retinal cholinesterase activity. Szepsenwol and Caretti ('42) reported that acetylcholine appears in the chick optic vesicles by the 5th day.

Other sites of cholinesterase activity in the early chick embryo are the somites, amnion, foregut, hindgut and heart. The function of this enzyme in these sites is as obscure as the early appearance of acetylcholinesterase in the nervous system, lens and notochord. Additional studies are needed to trace the fate of acetylcholinesterase in these sites beyond the 96-hour stage.

A second enzyme, slightly sensitive to cold acetone and physostigmine is present in the extra-embryonic membranes of the developing chick embryo. This enzyme, which is either lipase or more probably, aliesterase, first appears as a con-

stant finding in the area opaca of 21-hour embryos. As development proceeds, the highest enzymatic activity becomes localized in the yolk sac endoderm intimately associated with yolk globules. The enzyme is apparently the enzyme demonstrated by Buño and Mariño ('52) who used the Gomori "lipase" method ('45). That this method demonstrates aliesterase and not lipase as claimed, has been shown by Nachlas and Seligman ('49b). Buño and Mariño ('52) were first able to demonstrate "lipase" activity in the vitelline endoderm of three-day chicks. After the third day, enzymatic activity was demonstrable in the fore- and midgut, hepatic rudiment (three days), pancreatic bud (4 days) and in the stomach glands. Mesenchymal cells in the perichordal sheath of the endocardial cushions were also reactive as well as certain areas of precartilage. The method used in the present study should show enzymatic activity in all the sites demonstrated by these authors as well as acetylcholinesterase activity in addition. The Gomori procedure used by Buño and Mariño ('52) could not demonstrate cholinesterase activity. Enzymatic activity in the fore- and hindgut, heart, and slight activity in the hepatic rudiment were seen in the present study in fresh frozen sections but not in acetone-fixed embryos. Enzymatic activity in precartilage and pancreatic bud were not detected when 6-brom-2-naphthyl acetate was used as the substrate in either fresh frozen or acetone-fixed embryos. These differences may be due to low enzymatic activity in these sites in the early embryos studied or to differences in substrate specificity.

Ammon and Schütte ('34) have reported that low concentrations of cholinesterase occur in the yolk sac endoderm. These authors report that lipase activity increases early during the development of the yolk sac endoderm and later decreases as development proceeds. Yolk sac esterase does not show the same increase as lipase. Ammon and Schütte ('34) relied upon the hydrolysis of tributyrin as an indicator of lipase activity. However, recent work by Ravin and Seligman ('53) indicate that this substrate does not fulfill the

criteria for a high degree of specificity. Studies by Huggins and Moulton ('48) and others show that 4 C glycerides are attacked by aliesterase. Thus, the lipase activity in the yolk sac endoderm described by Ammon and Schütte ('34) and by Buño and Mariño ('52) may well be due to aliesterase or a mixture of aliesterase and lipase.

It is of interest that esterolytic activity in the yolk sac endoderm appears in the 21–36-hour chick embryo at a time when the vascular system is being completed. By the end of the second day the circulation is established. Thus, the esterolytic enzyme of the peripheral membranes develop concurrently with the vascular system which can then carry the products of yolk digestion to the developing embryo. According to Romanoff ('52), endodermal cells of the yolk sac secrete enzymes into the yolk. The cells then absorb the liquefied material and continue its digestion. Konopacka ('33) reports that yolk is an emulsion of fat droplets dispersed in a solution of colloidal phosphoproteins and Grodzinski ('47) has shown that lipases dissolve the yolk sphere membranes and convert fats from glycerides to phosphatides. Remotti ('30) reports that lipolytic activity is low until the 5th day, increases on the 10th day and is maximal on the 14th or 15th day. Studies by Ammon and Schütte ('34) show a similar sequence. Thus, the histochemically demonstrable esterolytic activity in the area opaca and yolk sac endoderm is apparently associated with yolk digestion. It appears that the metabolic demands of the developing embryo are met by the use of carbohydrate during the first 4 days of development (Spratt, '48, '49) and that the utilization of lipid develops later as the enzyme activity of the yolk sac endoderm increases.

SUMMARY

1. The development of esterolytic activity in fresh frozen and acetone-fixed chick embryos of 0–96 hours' incubation age has been studied by a series of histochemical methods.

2. An acetone and physostigmine-sensitive enzyme occurs in the embryonic axis and an acetone and physostigmine-

resistant enzyme is present in the extra-embryonic membranes.

3. The first enzyme appears to be acetylcholinesterase and the second lipase, or more probably, aliesterase.

4. The earliest acetylcholinesterase activity is detectable at the 15th hour stage, in the region of Hensen's node and in the primitive streak. The earliest aliesterase first appears as a constant finding in the area opaca at 21 hours.

5. Acetylcholinesterase activity can be detected in the developing nervous system of the chick at a time before morphological differentiation of neuroblasts has occurred. Also, this enzyme is present before its substrate, acetylcholine, can be detected in the embryo.

6. The subsequent patterns of the two enzymes are traced through the 96-hour embryo.

The author wishes to express his gratitude to Dr. George B. Wislocki for the making this work possible and for his encouragement throughout the course of this investigation. It is also a pleasure to acknowledge the careful work done by Mr. Alvin Shemish in preparing the diagrams of enzyme activity distribution.

LITERATURE CITED

ALLES, G. A., AND R. C. HAWES 1942 Cholinesterase in the blood of man. J. Biol. Chem., *133:* 375–390.

AMMON, R., AND E. SCHÜTTE 1934 Über das Verhalten von Enzymen im Hühnerei während der Bebrütung. Biochem. Zeitsch., *274–275:* 216–233.

AUGUSTINSSON, K. B. 1948 Cholinesterases, a study in comparative enzymology. Acta Physiol. Scand., *15:* Suppl. 52, 1–182.

BODINE, J. H. 1941 Enzymes in ontogenesis (Orthoptera). XX. The site of origin and distribution of protyrosinase in the developing egg of a grasshopper. J. Exp. Zool., *88:* 343–351.

BOELL, E. J., AND D. NACHMANSOHN 1940 Localization of cholinesterase in nerve fibers. Science, *92:* 513–514.

BOELL, E. J., AND S. C. SHEN 1944 Functional differentiation in embryonic development: I. Cholinesterase activity of induced neural structures in Amblystoma punctatum. J. Exp. Zool., *97:* 21–41.

———— 1950 Development of cholinesterase in the central nervous system of Amblystoma punctatum. J. Exp. Zool., *113:* 583–599.

BUÑO, W., AND R. G. MARIÑO 1952 Location of lipase activity in the chick embryo. Acta Anatomica, *16:* 85–92.

COONS, A. H., E. H. LEDUC AND M. H. KAPLAN 1951 Localization of antigen in tissue cells. VI. The fate of injected foreign proteins in the mouse. J. Exp. Med., *93:* 173–188.

DUVAL, M. 1889 Atlas d'embryologie. Masson. Paris.

EASSON, L. H., AND E. STEDMAN 1937 The specificity of choline-esterase. Biochem. J., *31:* 1723–1729.

GALLERA, J. 1948 Sur la distribution des substances basophiles cytoplasmiques dans le blastoderme de la Poule. Rev. Suisse. Zool., *55:* 243.

GOMORI, G. 1945 Microtechnical demonstration of sites of lipase activity. Proc. Soc. Exp. Biol. and Med., *58:* 362–364.

GRODZINSKI, Z. 1947 Bull. inter. acad. polon sci., Ser. B II: 169. Cit. Romanoff, A. L., 1952.

HUGGINS, C., AND S. H. MOULTON 1948 Esterases of testis and other tissues. J. Exp. Med., *88:* 169–179.

HUNTER, R. L. 1951 Distribution of esterase in the mouse embryo. Proc. Soc. Exp. Biol. and Med., *78:* 56–57.

KONOPACKA, B. 1933 Etude microchimique du comportement de la graisse dans le processus de formation du vitellus et dans le développement de l'embryon de Poule. Arch. biol., *44:* 251–305.

KUO, Z. Y. 1932 Ontogeny of embryonic behavior in aves. I. The chronology and general nature of the behavior of the chick embryo. J. Exp. Zool., *61:* 395–430.

———— 1938 Ontogeny of embryonic behavior in aves. XII. Stages in the development of physiological activities in the chick embryo. Am. J. Psychol., *51:* 361–379.

———— 1939 Studies on the physiology of the embryonic nervous system. IV. Development of acetylcholine in the chick embryo. J. Neurophysiol., *2:* 488–493.

LAZAROW, A., AND S. J. COOPERSTEIN 1953 Studies on the enzymatic basis for the Janus green B staining reaction. J. Histochem. and Cytochem., *1:* 234–241.

LEVY, M., AND H. PALMER 1940 Chemistry of the chick embryo. II. Weight, nitrogen and dipeptidase accumulation. J. Biol. Chem., *136:* 415–423.

———— 1943 Chemistry of the chick embryo. IV. Aminopeptidase. J. Biol. Chem., *150:* 271–279.

LINDEMAN, V. F. 1947 The cholinesterase and acetylcholine content of the chick retina, with especial reference to functional activity as indicated by the pupillary constrictor reflex. Am. J. Physiol., *148:* 40–44.

LILLIE, F. R. 1929 The development of the chick. Henry Holt and Co., New York.

MENDEL, B., D. B. MUNDELL AND H. RUDNEY 1943 Studies on cholinesterase. 3. Specific tests for true cholinesterase and pseudocholinesterase. Biochem. J., *37:* 473–476.

MOOG, F. 1943a Cytochrome oxidase in early chick embryos. J. Cell. and Comp. Physiol., *22:* 223–231.

MOOG, F. 1943b The distribution of phosphatase in the spinal cord of chick embryos of one to eight days incubation. Proc. Nat. Acad. Sci., *29:* 176–183.

———— 1944 Localizations of alkaline and acid phosphatase in the early embryogenesis of the chick. Biol. Bull., *86:* 51–80.

———— 1952 The differentiation of enzymes in relation to the functional activities of the developing embryo. Ann. N. Y. Acad. Sci., *55:* 57–66.

NACHLAS, M. M., AND A. M. SELIGMAN 1949a Histochemical demonstration of esterase. J. Nat. Cancer Inst., *9:* 415–425.

———— 1949b Evidence for the specificity of esterase and lipase by the use of three chromogenic substrates. J. Biol. Chem., *181:* 343–355.

NACHMANSOHN, D. 1939a Cholinesterase in voluntary muscle. J. Physiol., *95:* 29–35.

———— *1939b Cholinesterase dans le système nerveux central.* Bull. Soc. Chim. Biol., *21:* 761–796.

———— 1940 Cholinesterase in brain and spinal cord of sheep embryos. J. Neurophysiol., *3:* 396–402.

RAVIN, H. A., KWAN-CHUNG TSOU AND A. M. SELIGMAN 1951 Colorimetric estimation and histochemical demonstration of serum cholinesterase. J. Biol. Chem., *191:* 843–857.

RAVIN, H. A., AND A. M. SELIGMAN 1953 Determinants for the specificity of action of pancreatic lipase. Arch. Biochem. and Biophys., *42:* 337–354.

RAVIN, H. A., S. I. ZACKS AND A. M. SELIGMAN 1953 The histochemical localization of acetylcholinesterase in nervous tissue. J. Pharm. and Exp. Therap., *107:* 37–53.

RAWLES, M. E. 1936 A study in the localization of organ-forming areas in the chick blastoderm of the head process stage. J. Exp. Zool., *72:* 271–315.

REMOTTI, E. 1930 Sull assunzione delle riserve grasse durante lo sviluppo embryonale del pollo. Richerche Morph., *10:* 1. Cit. Romanoff, A. L., 1952.

RICHTER, D., AND P. G. CROFT 1942 Blood esterases. Biochem. J., *36:* 746–757.

ROMANOFF, A. L. 1952 Membrane growth and function. Ann. N. Y. Acad. Sci., *55:* 288–301.

RUDNICK, D. 1944 Early history and mechanics of the chick blastoderm. Quart. Rev. Biol., *19:* 187–212.

———— 1948 Prospective areas and differentiation potencies in the chick blastoderm. Ann. N. Y. Acad. Sci., *49:* 761–772.

RULON, O. 1935 Differential reduction of Janus green during development of the chick. Protoplasma, *24:* 346–364.

SAWYER, C. H. 1943a Cholinesterase and the behavior problem in Amblystoma. I. The relationship between the development of the enzyme and early motility. II. The effects of inhibiting cholinesterase. J. Exp. Zool., *92:* 1–29.

———— 1943b Cholinesterase and the behavior problem in Amblystoma. III. The distribution of cholinesterase in nerve and muscle throughout development. IV. Cholinesterase in nerveless muscle. J. Exp. Zool., *94:* 1–31.

SPRATT, N., JR. 1948 Development of the early chick blastoderm on synthetic media. J. Exp. Zool., *107:* 39–64.

SPRATT, N., JR. 1949 Nutritional requirements of the early chick embryo. I. The utilization of carbohydrate substrates. J. Exp. Zool., *110:* 273–298.

———— 1951 Demonstration of spatial and temporal patterns of reducing enzymes in early chick blastoderms by neotetrazolium chloride, potassium tellurite and methylene blue. Anat. Rec., *109:* 384–385.

———— 1952 Location of prospective neural plate in the early chick blastoderm. J. Exp. Zool., *120:* 109–130.

SZEPSENWOL, J., AND J. A. CARETTI 1942 Acetilcolina en embryones de pollo. II. Sus variaciones en las diferentes centros nerviosos durante el desarrollo. Rev. Arg. de Biol., *18:* 532–538.

TAHMISIAN, T. N. 1943 Enzymes in ontogenesis: Cholinesterase in developing Melanoplus differentialis eggs. J. Exp. Zool., *92:* 199–213.

WELSH, J. H., AND S. I. ZACKS 1949 Concerning the significance of the affinity of motor end-plates for Janus green B. Anat. Rec., *105:* 526 (abstract).

WENGER, B. S. 1951 Cholinesterase activity in different spinal cord levels of the chick embryo. Fed. Proc., *10:* 268–269.

WINDLE, W. F., AND D. W. ORR 1934 The development of behavior in chick embryos: spinal cord structure correlated with early somatic motility. J. Comp. Neur., *60:* 287–307.

YOUNGSTROM, K. A. 1938 On the relation between cholinesterase and the development of behavior in amphibia. J. Neurophysiol., *1:* 357–363.

———— 1941 Acetylcholinesterase concentration during the development of the human fetus. J. Neurophysiol., *4:* 473–477.

ZACKS, S. I. Unpublished observations.

ZACKS, S. I., AND J. H. WELSH 1951 Cholinesterases in rat liver mitochondria. Am. J. Physiol., *165:* 620–623.

———— 1953 Cholinesterase and lipase in the amoebocytes, intestinal epithelium and heart muscle of the quahog, Venus mercenaria. Biol. Bull., *105:* 200–211.

MALFORMATIONS OF THE TRUNCUS ARTERIOSUS IN PIG EMBRYOS

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THIRTY-FIVE FIGURES

This paper presents the results of a study of the malformations of the truncus arteriosus as they appear in pig embryos equivalent in age to human embryos of the third month of gestation. My purpose is to review and appraise current explanations of such anomalies. For this purpose abnormal pig embryos of from 18- to 50-mm length afford a new approach to an old problem. They are just old enough to show definite heart abnormalities, but still young enough to retain embryonic landmarks. An abnormality can be studied in its original setting.

MATERIALS AND METHODS

To date I have examined more than 50,000 pig embryos and have recovered 100 definitely abnormal embryonic hearts, nearly all equivalent to the third human month of development. My procedure is to open the thorax of the embryo and to examine the heart under a low power lens for gross abnormalities of the ventricles and great vessels. The method has its limitations; venous anomalies must often be missed, as well as internal defects that do not affect the external form. Suspected hearts, with the lungs and thorax are then sketched and cut into serial sections. The detailed study of any heart begins with a wax reconstruction.

OBSERVATIONS

Normal division of the truncus arteriosus. Many have exhausted their literary skill and the reader's imagination de-

scribing the division of the truncus arteriosus into aorta and pulmonary trunk. I cannot add any new facts, but I wish to emphasize certain aspects of the normal story that help in understanding abnormal hearts. The reader of the following will note resemblances to the more elaborate interpretation of Pernkoff and Wirtinger ('33).

In the beginning, the embryo heart ends in a long slender conus that extends to the dome of the pericardial sac (figs. 1 and 4). The conus ends as it perforates the pericardial sac; its distal end is soon marked by 4 distal endocardial cushions (fig. 2). The cushions are the source material for the future semilunar valves. The endocardial cushions lie just inside the pericardial envelope. Outside the pericardial envelope the conus continues into an irregular vascular sac. This is

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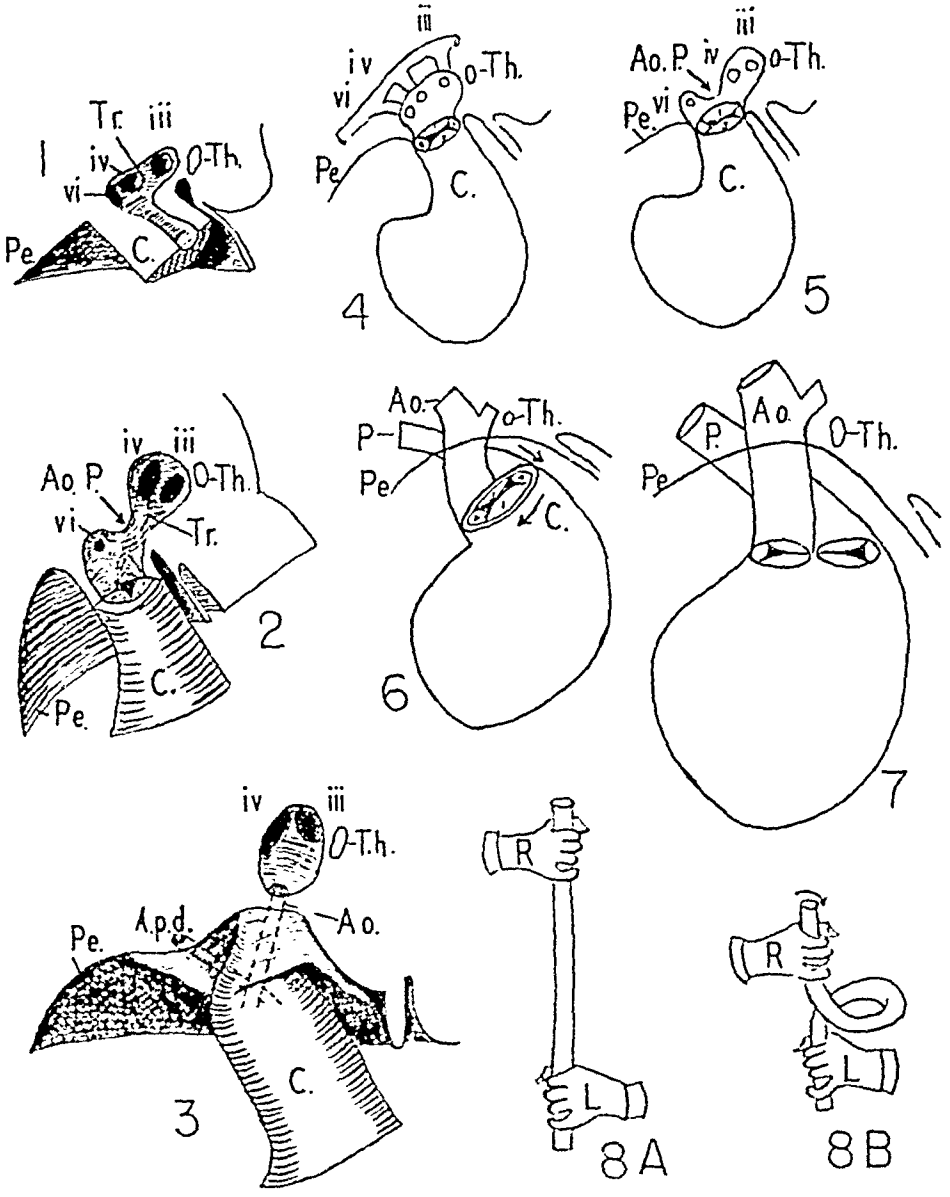
ABBREVIATIONS (APPLYING TO ALL FIGURES)

1, 2, 3, 4, Distal conus cushions
III, IV, VI, Arterial arches
+, Lower limit of aortico-pulmonary artery
=, Level of the distal conus cushions and semilunar valves
Ao.c., Coarctation of aorta
A.p.d.,s., Right and left pulmonary artery
Ao.d.,s., Right and left aorta
Ao.P., Aortico-pulmonary septum
A.V.d.,s., Right and left atrioventricular valve
Br.d.s., Right and left brachiocephalic artery
C., Conus
C.c.d.,s., Right and left common carotid artery
Co.d.,s., Right and left coronary artery
D.a., Ductus arteriosus
E., Esophagus
F.i., Interventricular foramen
h., Horn of the aortico-pulmonary septum
P., Pulmonary trunk
Pe., Pericardium
R.p., Proximal conus ridge
Sc.d.,s., Right and left subclavian artery
Th., Thyroid
Tr., Truncus
Tra., Trachea
V., Vagus
x., Recurrent branch of left vagus



Figs. 1–8 Models and diagrams of embryo pig hearts to illustrate the normal development of the aorta and pulmonary trunk from the truncus arteriosus.

Fig. 1 Truncus arteriosus and conus of a 5-mm embryo, A.E.C. 184. × 22½.
Fig. 2 Truncus arteriosus and conus of an 8-mm embryo, A.E.C. 137. × 22½.
Fig. 3 Truncus arteriosus and conus of an 8.5 mm embryo, A.E.C. 148. × 22½.
Fig. 4 Semidiagram of a 7-mm heart. × 15.
Fig. 5 Semidiagram of an 8-mm heart. × 15.
Fig. 6 Semidiagram of a 10-mm heart. × 15.
Fig. 7 Semidiagram of a 14.5-mm heart. × 15.
Fig. 8 A method of illustrating the twisting of the conus.

the truncus arteriosus. From it arise the arterial arches (figs. 1 and 4). The truncus arteriosus sac lies just outside the pericardial cavity and is firmly embedded in neck mesoderm. The thyroid gland is a useful landmark for the early truncus.

As has been often said, the truncus arteriosus is divided into an aorta and a pulmonary trunk by an aortico-pulmonary septum. The nature, direction, extent, and limitation of the aortico-pulmonary septum must always be kept in mind if abnormal hearts are to be understood. The aortico-pulmonary septum is a crescentic fold of neck mesoderm which pushes down between the 4th and 6th arterial arches (figs. 2 and 5). It pushes down in the simple frontal plane. It stops when it meets the distal conus cushions 1 and 3. It thereby divides the truncus arteriosus sac into two parts: a dorsal part for a future pulmonary trunk, and a ventral part for an aorta. The active independent growth of the aortico-pulmonary septum comes early and is soon ended; the free edge of the septum has fused with the upper surfaces of the distal endocardial cushions in a 10-mm embryo.

The two fragments of the truncus arteriosus, thus divided by the aortico-pulmonary septum, are only the source material for the two great vessels. The vessels develop as the truncus fragments are drawn down into the pericardial cavity and twisted about each other by two changes within the conus beneath. In the first place, the conus shortens; between the 7- and the 14-mm stages the distal cushions are pulled down on to the base of the ventricles (figs. 4 to 7). In the second place the distal end of the conus and its cushions twist nearly a half circle. The twisting can be illustrated if one takes a rubber tube and grasps it firmly as in figure 8 a. If one now forces the tube into the heart-tube shape as in figure 8 b, and then releases the grip of the right hand, the upper end of the rubber tube will rotate dextrally as does the heart conus. Of course the heart is not a rubber tube, but some such twisting — or rather untwisting for relief of stored up tension — does take place. The combined shortening of the conus and the rotation of its distal end draws out the

two truncus fragments into the spirally twisted aorta and pulmonary trunk (figs. 1 to 3 and 4 to 7).

The coronary arteries bud off from the aortic fragment of the truncus arteriosus just above the distal conus cushions. The left artery appears in a 10-mm embryo, the right in an 11-mm embryo. The origins of these vessels are naturally affected by rotation and shrinking of the conus beneath.

While the conus is drawing out and twisting the great vessels above it, it also undergoes internal transformation. As has been said, the distal end of the conus contains 4 endocardial cushions, two large and two small. After the aorticopulmonary septum fuses with the two larger cushions in the 10-mm stage the larger cushions fuse and then divide transversely. There are now 6 cushions, two sets of three each. In the 12-mm embryo each cushion begins a slow transformation into a semilunar valve cusp. The shrinkage of the conus brings the valves down upon the base of the ventricles. In addition there are two long spiral ridges which run the full length of the conus and partly divide its lumen. They are striking features of the early conus, but have nothing like the importance often attributed to them. They have nothing to do with the great vessels; they lie on the opposite side of the distal conus cushions. When the conus shrinks and twists the spiral ridges shorten and untwist and are reduced to two small endocardial pads. One is fixed in front of the interventricular foramen; the other is attached to the tricuspid valve ring behind the interventricular foramen. After the larger distal cushions have fused, the proximal ridge remnants fuse to divide the conus inlet. Much of their endocardial tissue is replaced by muscle; what is left constitutes the membranous septum. The details of the formation of the inlets of the aorta and pulmonary trunk I have given elsewhere (Shaner, '49). The proximal spiral conus ridges contribute to the heart and not to the great vessels.

If the study of abnormal hearts is to rise above simple description, one must proceed upon a hypothesis of some sort. In this study I have assumed that the chief cause for

heart anomalies is an arrest in development. In the normal heart there is a chain of overlapping events, beginning with the descent of the aortico-pulmonary septum in the 8-mm embryo and ending with the closure of the interventricular foramen in the 18-mm embryo, each step touching off the next. An arrest in development may happen at any stage, with graver consequences in the earlier ones. After a slight arrest at any stage, the heart may resume a belated and irregular development. All this may be true of any organ; but the heart story is complicated by the fact that the heart is an active pump and that structural maldevelopments which are out of phase with hydrodynamic changes in the embryo may be still further modified in consequence.

Effective causes for simple arrest in development are now well established; e.g., rubella in man and many laboratory agents in animals. The real question is how adequate a simple arrest may be to explain all heart anomalies, and what positive deviations in growth or what revivals of forgotten phylogenetic structures can be expected. The reader may judge these matters from the data that follow.

Truncus arteriosus communis persistens. Should the aortico-pulmonary septum fail to appear, but the conus develop more or less normally notwithstanding, the undivided truncus would be drawn out and twisted as a persistent truncus communis. I have no embryonic example, and I doubt that a fully attested neonatal case is known; that of Klemke ('25) is a highly probable one.

Humphreys ('32) has reviewed the probable neonatal cases and listed her criteria for a true case. These may be rearranged and restated as follows:

(a) A single large trunk with no trace of division.
(b) Two pulmonary arteries arising from the truncus between the coronary arteries and the normal aortic branches.
(c) Two coronary arteries that arise from the single trunk just above the semilunar valve and with origins that may betray an incomplete twisting of the conus.

(d) A single semilunar valve with 4, or perhaps three cusps.
(e) An undivided truncus inlet overriding an open interventricular foramen.

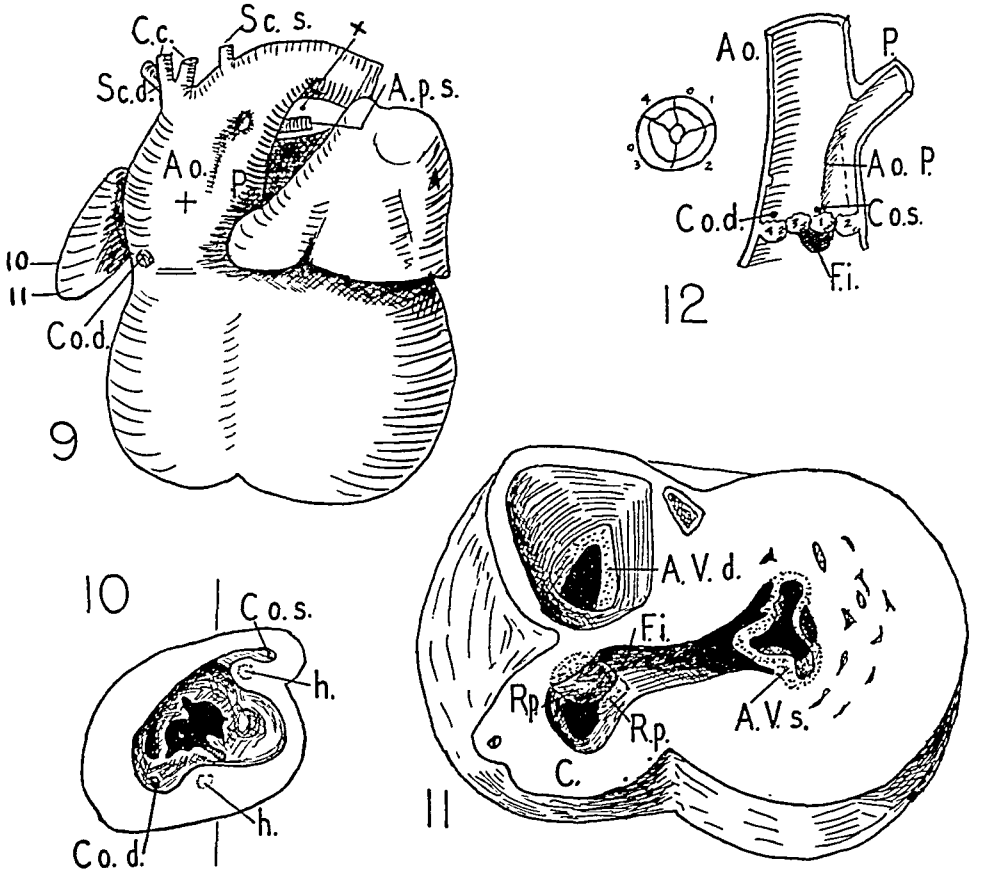
Criterion (a) is the key one. The rest show the results of the attempt of the conus to go on developing under the unusual conditions, and after an interruption. With (b) one should make certain that there is no common pulmonary vessel, which is the first product of an incipient aortico-pulmonary septum. The pulmonary arteries should also arise near the pericardial reflection, and considerably proximal to the left recurrent vagus nerve. A pulmonary artery that arises close to the nerve is probably using the ductus arteriosus as a secondary origin. Pulmonary vessels arising beyond the nerve are probably bronchial vessels. The coronary arteries (c) will develop from the undivided truncus but may fail to reach their normal positions if the twisting of the conus is incomplete. Theoretically, the single semilunar valve (d) should have 4 cusps, but one of the two smaller distal cushions does regress in partial truncus cases and may well do the same in truncus communis. Criterion (e) results from a lag in the last stage of conus development.

Nearly all published accounts of supposed truncus arteriosus communis persistens fail to mention the relation of the pulmonary vessels to the pericardium and to the left recurrent vagus nerve. A true truncus communis is very rare. Nearly every published case could be something else.

Partial truncus arteriosus. Partial truncus — partly divided into aorta and pulmonary artery — is a rather common find. Every grade of defective division is known, from a truncus with a mere suggestion of an aortico-pulmonary septum to one with the great vessels fully separated save for a small intercommunication just above the semilunar valves. An average example is that of Feller ('31), whose figure is reproduced as figure 12.

An embryonic case of partial truncus from a 35-mm pig embryo will show what has gone amiss in development. The

truncus of this heart (fig. 9) shows no external sign of division but a groove; internally the lumen is divided about halfway down. The free edge of the aortico-pulmonary septum is crescent-shaped; its horns have pushed ahead into the distal conus cushion mass (fig. 10). The coronary arteries spring from the undivided part of the truncus. Their plane of origin and that of the septal horns betray a rotation short of normal. The distal conus cushions (fig. 10) form an irregular



Figs. 9–12 Partial truncus arteriosus.

Fig. 9 Model of the heart of a 35-mm pig embryo, A.E.C. 841. The lower limit of the aortico-pulmonary septum is indicated by (+), and the level of the distal conus cushions and the coronary arteries by (=). × 12½.

Fig. 10 Section through the conus of the same heart, to show the distal conus cushions and the coronary arteries. × 12½.

Fig. 11 Section through the conus to show proximal conus ridges and interventricular foramen. × 12½.

Fig. 12 Neonatal case of partial truncus arteriosus, from Feller ('31).

ring of undeveloped or regressing endocardial tissue. Beneath the distal cushion ring are the remains of the proximal spiral ridges (fig. 11). These have been untwisted, foreshortened, and reduced to pads. They have failed to fuse and to divide the conus inlet. The rest of the heart is normal for the stage.

Taken as a whole, such a heart is a fair picture of what would result from a simple arrest in development at the 9- to 10-mm stage, followed by a nearly normal resumption of growth. The later development of the atria, of the atrioventricular valves, and of the ventricles went on normally, even the conus shortened and twisted almost normally. But the early arrest blunted the aortico-pulmonary septum so that it could not divide the truncus, nor initiate the normal chain of transformations within the distal and proximal endocardial conus tissue masses.

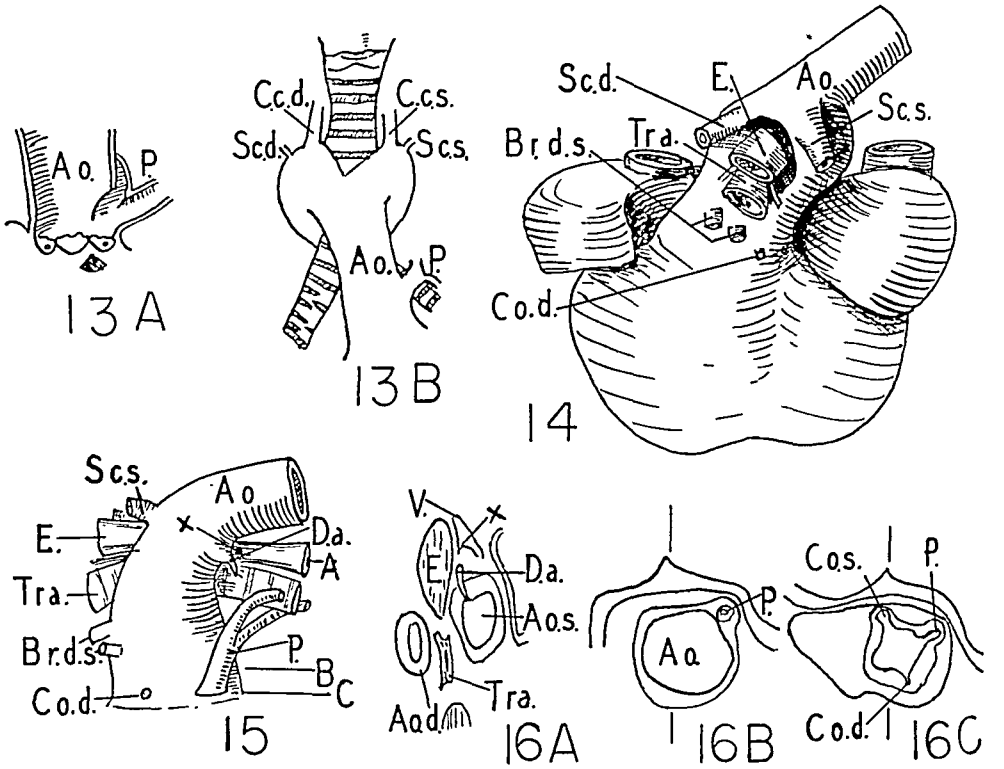
Vierordt (1898) long ago suggested that one might expect three distinct varieties of partial truncus arteriosus:

(a) Partial truncus with the short aorta and pulmonary trunk each of normal caliber — the kind just discussed.
(b) Partial truncus aorticus, with the aorta predominant and the pulmonary trunk reduced in caliber.
(c) Partial truncus pulmonalis, with the aorta reduced in caliber and the pulmonary trunk enlarged, the trunk with the ductus arteriosus constituting the main outlet from the heart.

The second and third varieties correspond to pulmonary and aortic stenosis in hearts with a completely divided truncus.

Partial truncus aorticus. A neonatal example of partial truncus aorticus combined with a double aortic arch has been described by Kerwin ('36). His figures are reproduced as figure 13. The short undivided part of the truncus begins with a tricuspid semilunar valve. There are two coronary arteries. A low arching ridge represents the horns of an aortico-pulmonary septum that separates a slender pulmonary trunk from the much larger aorta.

A 30-mm pig embryo heart that duplicates Kerwin's specimen is shown in figures 14, 15, and 16. The heart is normal save for its conus and truncus. The endocardial tissue of the conus has disappeared, so there are no semilunar valves. The coronary arteries mark the beginning of the truncus.



Figs. 13–16 Partial truncus arteriosus aorticus.

Fig. 13 Neonatal case of partial truncus arteriosus aorticus after Kerwin ('36).

Fig. 14 Model of the heart of a 30-mm pig embryo, A.E.C. 1050, × 7½.

Fig. 15 Side view of the conus of the same. × 7½.

Fig. 16 Three sections through the conus, at levels indicated in figure 15. × 7½.

They show incomplete rotation (fig. 16 c). The undivided truncus is very short, and the slender pulmonary trunk arises at the level of the coronary arteries (figs. 15 and 16 c). The oversized aorta leads into two aortic arches, each furnished with a small brachiocephalic and a large subclavian branch. The aortic arches enclose the two vagi, the esophagus, and

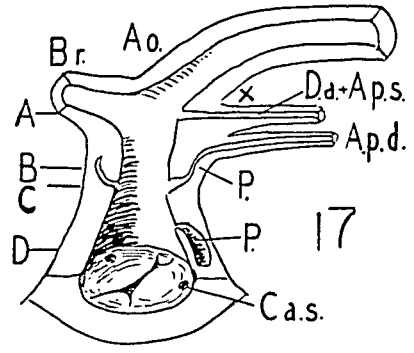
the trachea; definitely constricting the two tubes. The small pulmonary trunk ends in two normal pulmonary arteries. The ductus arteriosus has degenerated precociously, leaving only a tag attached to the left aortic arch (fig. 15). There is a wide open interventricular foramen.

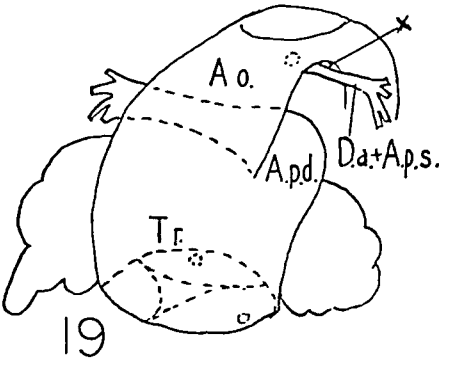
The 30-mm embryo heart repeats the general picture of partial truncus, but complicated by a double aortic arch and the atrophy of the pulmonary trunk and ductus arteriosus. There may be a causal connection between the added defects. The double arch gives the aorta an advantage, enabling it to drain blood from the pulmonary trunk. The pulmonary trunk and ductus arteriosus would thus be deprived of their normal internal stimulus and would regress, as do unused vessels elsewhere in the body.

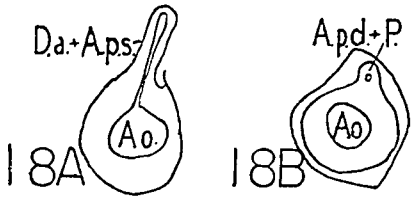
The causes that produce partial truncus aorticus might go farther, and produce a secondary breakdown of the aorticopulmonary septum. The 40-mm embryo, whose description follows, is an example.

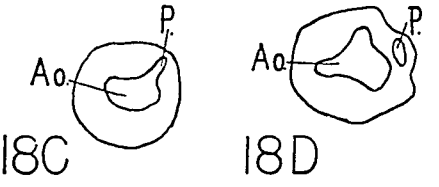
This heart would seem at first glance (fig. 17) to be a case of truncus arteriosus communis. A large single arterial trunk arises from the right ventricle over an open interventricular foramen. It begins with 4 imperfect semilunar cusps, one of which is rudimentary. Above the cusps arise the two coronary arteries. Beyond the coronary arteries there are some extraordinary features. The main vessel has all the characters of an aorta. The left pulmonary artery (figs. 17 and 18 a) arises close to the left recurrent vagus by a root that has all the characters of a ductus arteriosus. The root of the right pulmonary artery has the course and relations of a segment of a much reduced pulmonary trunk (figs. 17 and 18 b and c). This fragment of the pulmonary trunk arises high above the coronary arteries; proximal to it and in line with it there is a blind sac which has the position and character of another segment of the pulmonary trunk (figs. 17 and 18 d). The blind sac ends over the left coronary artery.

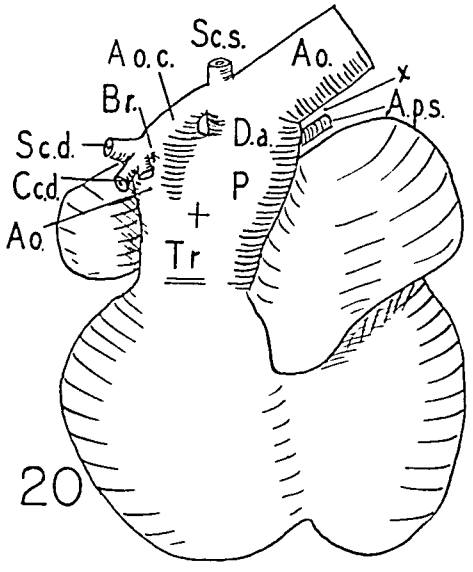
The whole arrangement is another case of partial truncus aorticus complicated with considerable secondary degenera-











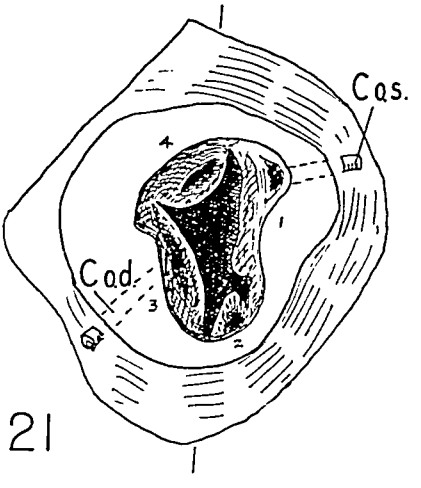


Fig. 17 Graphic reconstruction of the conus of a 40-mm pig embryo, A.E.C. 1009. × 7½.

Fig. 18 Sections through the same conus at levels indicated in figure 17. × 7½.

Fig. 19 Conus of a 35 year old heart after MacGilpin ('50).

Fig. 20 Model of a 42-mm pig embryo heart, A.E.C. 1031. An example of partial truncus arteriosus pulmonalis. The lower limit of the aortico-pulmonary septum is marked by (+), the level of the distal conus cushions by (=). × 7½.

Fig. 21 Section through the conus of the same heart, to show the distal conus cushions and coronary arteries. × 15.

tion of the pulmonary pathway. Had the embryo lived the proximal vestigial sac would likely have disappeared altogether. The smallest semilunar cusp would also have dropped out. The resulting heart would then have exactly duplicated the unusual heart from a 35 year old woman described by MacGilpin ('50), and reproduced as figure 19. MacGilpin interpreted his heart as a case of truncus communis persistens.

A secondary perforation of the aortico-pulmonary septum may occur in otherwise normal hearts. Such a communication normally occurs between the two aortae of the crocodile, where it is known as the foramen of Panizza. Hochstetter ('06) considers the foramen a secondary perforation. A somewhat similar foramen occasionally develops between the aorta and pulmonary trunk in man. Gross ('52) has successfully closed one. Such defects in mammals must be distinguished from an imperfectly descended septum, for the septum is intact proximal to the foramen and is properly attached to the distal conus cushions and semilunar valves.

Partial truncus pulmonalis. The third variety of partial truncus with the pulmonary trunk and ductus arteriosus predominating over the aorta is rare. Vierordt (1898) knew of no examples. Humphreys ('32) recognized two: a heart described by Feller ('31) reproduced in figure 27, and another by Preisz (1890) shown in figure 28.

I have three embryo hearts afflicted with this malformation in three degrees of gravity:

A.E.C. 1031, 42 mm, figures 20 and 21
A.E.C. 1008, 52 mm, figures 22 and 23
A.E.C. 1065, 30.5 mm, figures 24, 25, and 26

All three hearts have a partial truncus that leaves the right ventricle and divides about halfway up into an aorta and a pulmonary trunk. The distal conus cushions (figs. 21, 23, and 26) show various degrees of arrested development. The coronary arteries are normal, save for incomplete rotation of their origins. The proximal conus ridges are reduced to flat pads in the first heart, and are vestigial in the other two. The ventricles are normal except for the open interventricu-

lar foramena. The atria are normal in each case. So far each heart presents the familiar picture of a partial truncus that is the result of an arrest of the aortico-pulmonary septum followed by a halting resumption of development of the conus.

All three hearts are peculiar in that the pulmonary trunk and the ductus arteriosus are the functional beginning of the aortic arch, with the pulmonary trunk giving off the two pulmonary arteries in the normal fashion. The aorta is much reduced. In the 42-mm embryo (fig. 20) the small aorta gives rise to two common carotids and a right subclavian and then degenerates into a solid cord. The left subclavian arises distally from the aortic arch. In the 52-mm embryo heart (fig. 22) the solid segment of the preceding heart is absent altogether. The aortic stump gives rise to the common carotids, but the right subclavian comes off the remnant of the right aortic arch — an added abnormality. In the 30.5 mm embryo heart (figs. 24, 25, and 26) the aortic stump is still more reduced and deformed.

Feller's heart corresponds to my 42-mm heart. Compare figures 27 and 20. The Preisz heart (fig. 28) at first sight seems pathological as Preisz thought it, but if one makes allowance for the oversized pulmonary arteries, it corresponds quite well to my 30-mm embryo heart (fig. 24).

Two distinct causes must have combined to produce these cases of partial truncus pulmonalis. First, an early arrest

Fig. 22 Model of a 52-mm pig embryo heart, A.E.C. 1008. The lower limit of the aortico-pulmonary septum is indicated by (+), the level of the distal conus cushions by (=). × 7½.

Fig. 23 Section through the conus of the same, to show the distal conus cushions and the coronary arteries. × 15.

Fig. 24 Model of a 30.5-mm pig embryo heart, A.E.C. 1065. The lower limit of the aortico-pulmonary septum is indicated by (+), the level of the distal conus cushions by (=). × 7½.

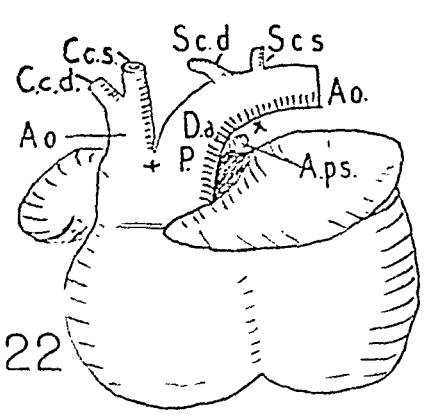
Fig. 25 Side view of the conus of the same heart. × 7½.

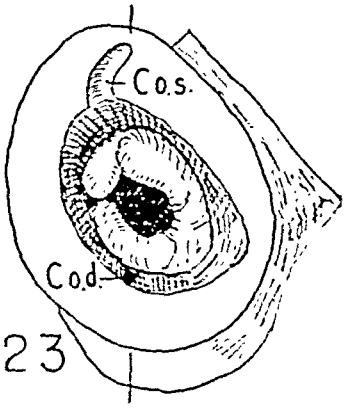
Fig. 26 Section through the conus of the same heart, to show the distal conus cushions and the coronary arteries. × 15.

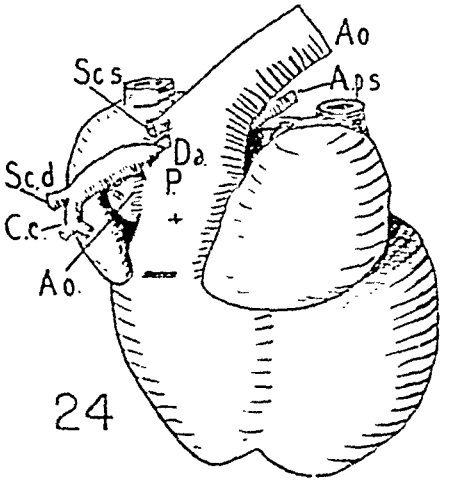
Fig. 27 A neonatal case of partial truncus arteriosus pulmonalis. After Feller ('31).

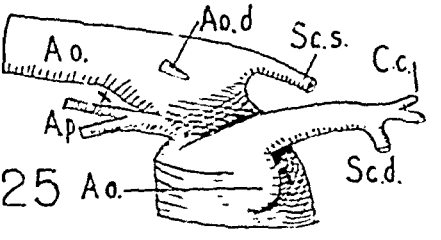
Fig. 28 A neonatal case of partial truncus arteriosus pulmonalis. After Preisz (1890).

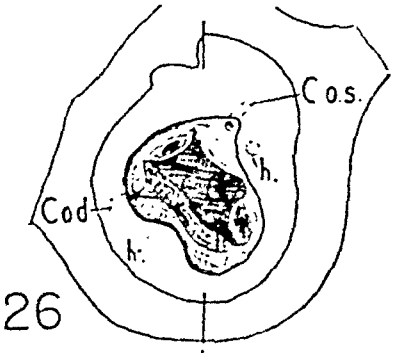
at around the 8-mm stage produced partial truncus. Then, at about the 12-mm stage, fetal coarctation of the aorta set in. The second cause forced the pulmonary trunk and the ductus arteriosus to become the main outlet of the heart.

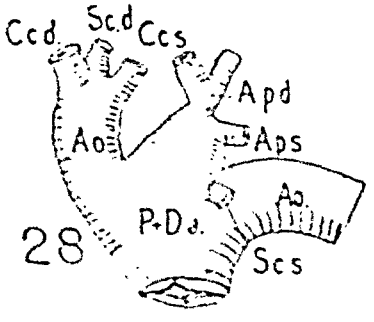


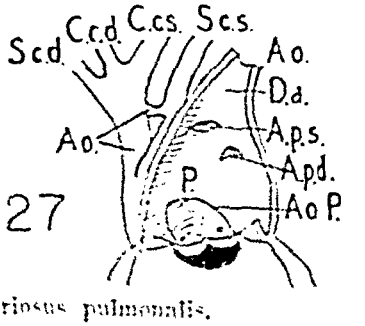












Figs. 22–28 Partial truncus arteriosus pulmonalis.

Bremer ('48) gives evidence that fetal coarctation is caused by an overgrowth of endothelial tissue at the point where the lost segment of the dorsal aorta joins the 4th arterial arch. The overgrowth would obstruct the lumen of the 4th arch and cause coarctation. It would also stop the normal upward migration of the left subclavian artery and leave it stranded on the aortic arch, as it is in my three embryos and in the two neonatal cases.

False forms of truncus arteriosus. No study of the truncus arteriosus is complete without a consideration of vessel patterns that mimic it. Writers have often misunderstood cases of extreme aortic and pulmonary stenosis — such as may follow normal and complete division of the truncus — and have called them some form of persistent truncus. To distinguish each is not always easy.

Ordinary extreme aortic stenosis may result in a set of great vessels that simulate partial truncus pulmonalis. The vessels of a 25-mm pig embryo heart shown in figure 29 might be so interpreted. But a careful examination of the aorta will bring out that it was once fully formed. There will be found some trace of a separate origin of the aorta from the ventricle, and vestiges of normally formed semilunar cusps. Above all the coronary arteries will be found to arise from the aorta and not from the supposed undivided truncus. Indeed the aorta seems to be retained to supply the coronary arteries by blood flow in the reversed direction.

Peacock (1866) reviewed the earlier examples of this sort; he seems to have thought them forms of undivided truncus. Konstantinowich ('06) correctly interpreted them as ordinary extreme aortic stenosis. Yet Ostreicher and Harris ('50) recently describe a heart identical with one of Konstantinowich as "common truncus arteriosus."

On the other hand, ordinary pulmonary stenosis may simulate partial truncus arteriosus and even truncus communis persistens.

The common forms of pulmonary stenosis cause no confusion; the vessels of the 28-mm embryo heart shown in fig-

ure 30 are clearly derived from a fully divided truncus. But in more degenerated forms the proximal part of the pulmonary trunk may disappear, as in the 33-mm embryo heart shown in figure 31. The pulmonary trunk is now a withered stump that clings to the aorta, and the pulmonary arteries

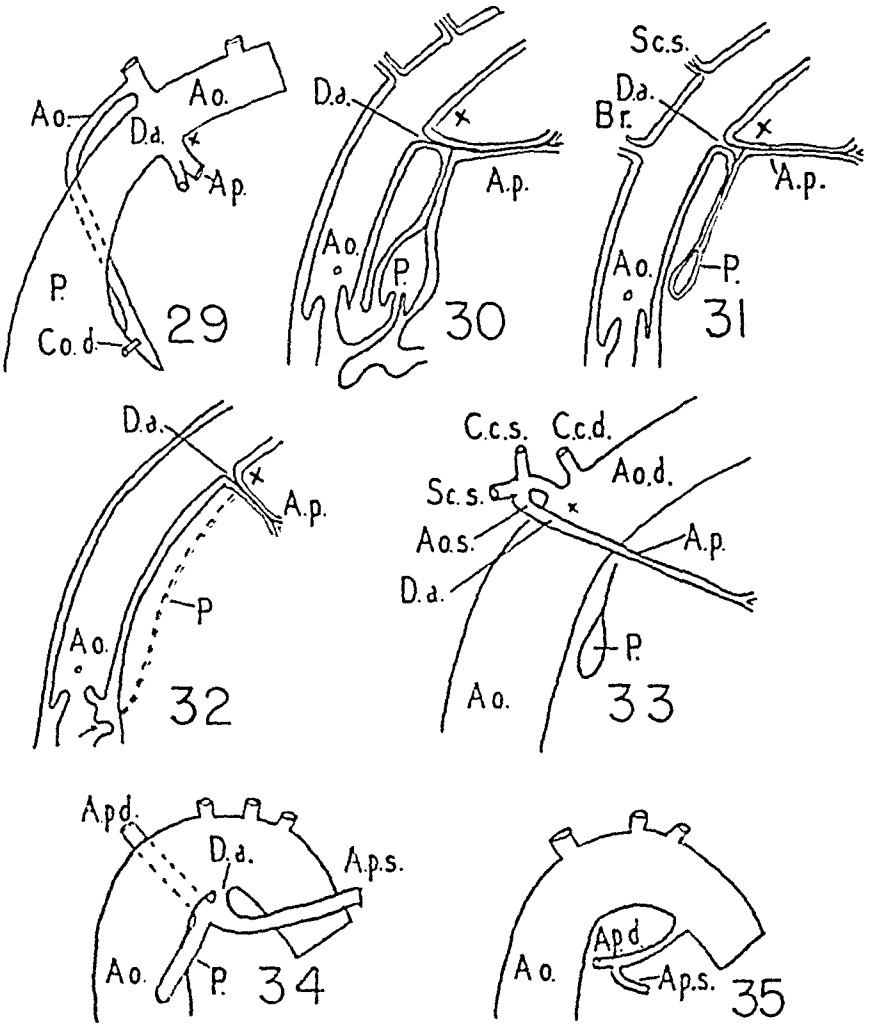


Fig. 29 Great vessels of a 25-mm pig embryo, A.E.C. 859.
Fig. 30 Great vessels of a 28-mm pig embryo, A.E.C. 1029.
Fig. 31 Great vessels of a 33-mm pig embryo, A.E.C. 1060.
Fig. 32 Great vessels of a 40-mm pig embryo, A.E.C. 820.
Fig. 33 Great vessels of a 50-mm pig embryo, A.E.C. 1055.
Fig. 34 "Truncus solitarius aorticus." After Kugel ('31).
Fig. 35 "Truncus arteriosus communis persistens." After Hunter ('44).

are supplied from a reversed-flow ductus arteriosus, which in turn may be identified by its relation to the recurrent vagus nerve. Kugel ('31) reports two neonatal hearts of this sort, one of which is reproduced in figure 34. Kugel does not mention the vagus, but the significance of the vessels is evident.

A further step in degeneration of the pulmonary trunk is shown in a 40-mm embryo heart (fig. 32). Here the entire pulmonary trunk is gone, save for a niche in the conus. The pulmonary arteries arise from a reversed-flow ductus arteriosus, again identified by its relation to the left recurrent vagus nerve. If the niche of this heart were lost and the vagus nerve not identified, the ductus arteriosus might be considered a 6th arterial arch springing from an undivided truncus. Two neonatal hearts of this sort are on record: one given by Hunter ('44) and a second by Webb ('46). A diagram of Hunter's case is given in figure 35. There is no reference to a niche, nor to the vagus, in either account. Both authors consider their hearts examples of truncus communis persistens. While this interpretation is possible, it should be remembered that true truncus is rare and pulmonary stenosis is common, and that both hearts are more likely examples of extreme pulmonary stenosis.

Greater uncertainty surrounds the "truncus communis persistens" cases of Hülse ('18) and Siegmund ('28). With Hülse's heart the lungs are supplied by two "bronchial" arteries that arise from a single root just distal to the left subclavian. In Siegmund's case there are numerous arteries to the lungs from the same general region. Which vessels are true pulmonary arteries, and which bronchial can only be guessed, for the position of the left recurrent vagus is not mentioned. Some of the vessels might well be true pulmonary arteries arising from a persisting ductus arteriosus. In general, when the solitary vessel from the heart has the character of an aorta, and the vessels to the lungs arise in the neighborhood of the recurrent vagus, extreme pulmonary stenosis is a likelier explanation.

As a further instance of the complexities one may expect, I add the reconstruction of the vessels of a 50-mm embryo heart in figure 33. They are another case of extreme pulmonary stenosis, with the remnant of the pulmonary trunk embedded in the wall of the ascending aorta. The aorta continues into a right-sided aortic arch. Only a distal remnant of the left aortic arch is retained to furnish origin for the left subclavian and the left ductus arteriosus. The latter in turn serves the normal pulmonary arteries. So far I have found no neonatal heart to match this embryo.

DISCUSSION AND GENERAL CONCLUSIONS

A detailed study of heart malformations in the embryo does not lend encouragement to those who prefer elaborate theories. The basic cause for every heart malformation given in this paper is an arrest in development of some sensitive growing part, followed by a halting general resumption of growth. In the heart this resumption may be modified by the hydrodynamic forces peculiar to an active vascular system. Growth *in excessu* of endocardial and endothelial tissue is sometimes a significant additional factor; e.g., in aortic stenosis (Shaner, '49) and in coarctation (Bremer, '48). Competition between parallel vessels may result in degeneration of the pulmonary trunk and perhaps the perforation of the aortico-pulmonary septum. Given a normal heart to begin with, a few well established factors can produce the most complex abnormalities, without recourse to phylogenetic revivals.

After all, the fundamental theory of Stockard ('21) for fish embryo malformations is still the best for all forms. The recent work with rubella, high altitudes, and various laboratory reagents does no more than supply specific agents which might affect mammalian embryos, and thus extends Stockard's theory to higher forms.

SUMMARY

1. The author has examined to date 50,000 pig embryos and has recovered 100 abnormal hearts, mostly in the 18- to 50-mm stages.

2. A critical review of the normal development of the truncus arteriosus is given, which stresses the short independent activity of the aortico-pulmonary septum, and the longer secondary effect upon the great vessels of the shortening and twisting of the conus beneath them.

3. A revised set of criteria for truncus arteriosus communis persistens is given.

4. A case of partial truncus arteriosus in an embryo is described in detail. The defect is explained as an arrest in the 8-mm embryo, followed by a halting or partial resumption of heart development.

5. A case of partial truncus arteriosus aorticus is described in detail. It is ordinary partial truncus complicated by pulmonary stenosis.

6. Several cases of partial truncus arteriosus pulmonalis are also presented. They are partial truncus complicated by aortic coarctation.

7. Several cases of ordinary aortic and pulmonary stenosis which simulate persistent truncus arteriosus are described. Neonatal cases of the same sort are discussed and criteria for the separation of such false cases are stated.

8. In general, a simple arrest in development, followed by a halting resumption of growth, complicated by hydrodynamic forces and other minor but well known disorders will explain the most complicated heart abnormalities.

I wish to record my great obligations to the Swift Canadian Company, Canada Packers Ltd., and to Burns and Company Ltd. for facilities so freely granted me to gather the embryos for this study; to Dr. R. L. Yanda, Mr. R. L. Sutherland and Dr. T. Shnitka, who as medical students collected the embryos; to the University of Alberta Medical Research Fund for grants-in-aid, and to Mr. A. G. Fairall who prepared the embryos for study.

LITERATURE CITED

BREMER, J. L. 1948 Coarctation of the aorta and the aortic isthmuses. Arch. Path., *45:* 425–434.

FELLER, A. 1931 Zur Kenntnis der angeborenen Herzkrankheiten. Truncus arteriosus communis persistens und seine formale Entstehung. Arch. f. path. Anat., *279:* 869–910.

GROSS, R. C. 1952 Surgical closure of an aortic defect. Circulation, *5:* 858–863.

HOCHSTETTER, F. 1906 Beiträge zur Anatomie und Entwicklungsgeschichte des Blutgefässsystems der Krokodile. Voltzkow's Reise in Ostafrika, *4:* 1–139.

HÜLSE, W. 1918 Beitrag zur Kenntnis der totalen Persistenz des Truncus arteriosus communis. Arch. f. path. Anat., *225:* 16–24.

HUNTER, O. B. 1944 Truncus arteriosus communis persistens. Arch. Path., *37:* 328–330.

HUMPHREYS, E. M. 1932 Truncus arteriosus communis persistens. Arch. Path., *14:* 671–700.

KERWIN, A. J. 1936 Persistent (partial) truncus arteriosus associated with double aortic arch. J. Tech. Meth. and Bull. Inter. Assoc. Med. Mus., *15:* 142–147.

KLEMKE, W. 1925 Ein klassiker Fall von totaler Persistenz des Truncus arteriosus communis. Centralblatt f. allg. Path. u. path. Anat., *36:* 307–312.

KUGEL, M. A. 1931 A clinical and pathological study of two cases of truncus solitarius aorticus. Am. Heart J., *7:* 262–273.

KONSTANTINOWICH, W. 1906 Prag. med. Wchschr., *49:* 657. From Bland et al., Am. Heart J., *8:* 787–801.

MACGILPIN, H. H. 1950 Truncus arteriosus communis persistens. Am. Heart J., *39:* 615–625.

OSTREICHER, D. L., AND G. E. HARRIS 1950 An unusual cardiac anomaly. Am. Heart J., *39:* 626–628.

PEACOCK, T. B. 1866 On the malformations of the human heart. London.

PERNKOPF, E., AND W. WIRTINGER 1933 Die Transposition der Herzostien — ein Versuch der Erklärung dieser Erscheinung. Zeitschr. f. Anat. u. Entwick., *100:* 563–711.

PREISZ, H. 1890 Beiträge zur Lehre von den angeborenen Herzanomalien. Ziegler's Beiträge z. path. Anat., *7:* 245–298.

SIEGMUND, H. 1928 Totale Persistenz des Truncus arteriosus communis. Zeitschr. f. Kreislaufforschung, *20:* 65–73.

SHANER, R. F. 1949 Malformation of the atrio-ventricular endocardial cushions of the embryo pig, and its relation to defects of the conus and truncus arteriosus. Am. J. Anat., *84:* 431–455.

———— 1951 Complete and corrected transposition of the aorta, pulmonary artery and the ventricles in pig embryos, and a case of corrected transposition in a child. Am. J. Anat., *88:* 35–62.

STOCKARD, C. S. 1921 Developmental rate and structural expression: an experimental study of twins, "double monsters" and single deformities, and the interaction among embryonic organs during their origin and development. Am. J. Anat., *28:* 115–277.

VIERORDT, H. 1898 Die angeborenen Herzkrankheiten. In Nothnagel, Handb. d. spezielle Pathologie u. Therapie, *15:* 132. Wien.

WEBB, A. C. 1946 Truncus arteriosus communis persistens. Arch. Path., *42:* 427–432.

THE ACTION OF EPINEPHRINE ON THE BLOOD VESSELS OF CORTISONE-TREATED ANIMALS [1]

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TWELVE FIGURES

There have been innumerable reports in the literature on the physiological and pharmacological effects of cortisone, both in human and animal investigations. This report presents an interesting aspect of cortisone action, which has not been mentioned in the literature, that may have some bearing in diseases of the vascular system. The vasoconstrictive action of epinephrine is a well known and documented phenomenon (Goodman and Gilman, '41). It is the purpose of this paper to report on the effect of epinephrine on the blood vessels of cortisone-treated hamsters.

METHODS

Two methods of study were employed. (1) Hamsters of approximately 120 gm body weight were injected intraperitoneally with 25 mg of cortisone acetate (Merck) for three consecutive days. Control animals were injected with 1 ml of sterile saline solution for the same period of time. Four to 6 hours after the last dose of cortisone, the animal was moderately anesthetized with nembutal (3 mg/100 gm body weight), one cheek pouch everted and pinned across a window in a cork board somewhat in the manner described by Fulton, Jackson and Lutz ('47). With transmitted light and a dissecting microscope at 60 diameter magnification the vessels were directly observed and measured with an ocular micrometer. While

[1] This investigation was supported (in part) by U.S. Public Health Grant H-1591.

under direct observation, the hamster was injected intraperitoneally with a dosage of epinephrine known to cause vasoconstriction in a normal untreated hamster (0.16 ml/100 gm body weight of 1 : 1000 epinephrine hydrochloride). Control animals were studied in the same manner. (2) Excised pieces of the cheek pouch tissue, or segments of femoral artery were taken

TABLE 1

NON-CORTISONE TREATED (Controls)			CORTISONE TREATED (Experimentals)		
Animal	Diam. of artery (microns)		Animal	Diam. of artery (microns)	
	Before epinephrine	After epinephrine		Before epinephrine	After epinephrine
1C	42	17	1E	58	42
2C	58	25	2E	33	25
3C	33	16.5	3E	33	17
4C	58	30	4E	25	16.5
5C	33	8.3	5E	50	33
6C	42	15	6E	42	33
7C	42	20	7E	41.5	34
8C	58	17	8E	33	25
9C	33	8.5	9E	58.5	41
10C	25	8.3	10E	42	33
11C	35	9	11E	31	23
			12E	52.5	34.5
			13E	27	18.5
			14E	42	38
Average	41.7	15.4	Average	40.6	29.5
Average % reduction: 63%			Average % reduction: 27%		

from both control and cortisone-treated hamsters. The exterior surface of the tissues were washed several times in warm mammalian Ringer's solution and gently teased out flat. The blood vessels were observed before and after adding 2 drops of 1 : 1000 epinephrine solution to the Ringer's solution bathing the excised tissue.

RESULTS

Table 1 summarizes the results. In the control group of 11 hamsters, that had received physiological saline solution intraperitoneally instead of cortisone, there was an average of 63%

reduction in the diameter of the arteries occurring within 3–5 seconds after the intraperitoneal injection of epinephrine. The vessels did not return to their original diameter for at least 30 minutes (table 1, figs. 1 and 2).

In contrast to the controls the experimental group of 14 hamsters pretreated with cortisone showed an average of 27% reduction in the diameter of the arteries (table 1). This reduction did not become evident until, at least, 5 minutes after the administration of epinephrine. Furthermore, the slight vasoconstrictive effect lasted only 10–12 minutes (figs. 3 and 4).

The in vitro studies were done as outlined in the second procedure. Figure 5 illustrates a darkened blood-filled artery from a control hamster cheek pouch before epinephrine was introduced in the solution bathing the tissue. Figure 6 shows this same artery one minute after adding 2 drops of epinephrine. The artery had markedly constricted and forced the blood cells from the cut ends of the vessel. A reduction in its diameter is also apparent. Following the same procedure with tissue from a cortisone-treated hamster, figures 7 and 8 show the relative ineffectiveness of epinephrine to constrict the artery. The vessel shows minimal constriction; retains its blood cells in its lumen and remains quite visible.

Using a muscular artery from another region of the animal, identical results were obtained. Figures 9 and 10 illustrate isolated segments of femoral artery before and after epinephrine application from a control hamster. The "X" in figure 10 points to regions of early constriction which becomes progressive along the vessel. Figures 11 and 12 are greatly enlarged views of an isolated segment of a femoral artery from a cortisone-treated animal. One can note a very slight reduction of arterial diameter in figure 12, the vessel having been photographed 5 minutes after epinephrine application.

DISCUSSION AND CONCLUSIONS

These experiments seem to indicate that cortisone in some manner altered or blocked the response of the smooth muscle

cells in the peripheral blood vessels to the action of epinephrine. The in vitro studies also show that blood vessels in excised pieces of tissue reacted similarly to those of the intact animals. Hence the author concludes that cortisone had modified the smooth muscle cell in its reaction to epinephrine. Other possible mechanisms of cortisone action must also be mentioned. There may have resulted an increase in the amount of amine oxidase or in its activity, thus hastening the breakdown of epinephrine. However, the in vitro experiments would cast doubt on the latter possibilities. The reaction time or the degree of contraction was not changed significantly on comparing the results of the in vivo and in vitro experiments.

How cortisone affects the smooth muscle cells of the peripheral blood vessels, in order to reduce their normal sensitivity to epinephrine, is still unknown. Cortisone also inhibits or reduces the formation of new blood vessels as demonstrated admirably by Ashton and Cook ('52). Whether there is any connection between cortisone/epinephrine action on the peripheral blood vessels and wound healing (Ragan et al., '49; Howes et al., '50) or the formation of new blood vessels remains to be investigated.

SUMMARY

Epinephrine administered in vasoconstrictive dosages failed to cause a marked decrease in arterial diameter in the cheek pouch and femoral arteries of hamsters pretreated with large doses of cortisone. Both in vivo and in vitro studies were performed. Some possible mechanisms of action of cortisone on the blood vessels relative to the epinephrine response are discussed.

LITERATURE CITED

ASHTON, N., AND C. COOK 1952 In vivo observations of the effects of cortisone upon the blood vessels in rabbit ear chambers. Brit. J. of Exp. Path., *33:* 445–450.

FULTON, G. P., R. G. JACKSON AND B. R. LUTZ 1947 Cinephotomicroscopy of normal blood circulation in the cheek pouch of the hamster. Science, *105:* 361–362.

GOODMAN, L., AND A. GILMAN 1941 The Pharmacological Basis of Therapeutics. The Macmillan Company, New York. Chap. 23, 399–401, 404–405.

HOWES, E. L., C. M. PLOTZ, J. W. BLUNT AND C. RAGAN 1950 Retardation of wound healing by cortisone. Surgery, *28:* 177–181.

RAGAN, C., E. L. HOWES, C. M. PLOTZ, K. MEYER AND J. W. BLUNT 1949 Effect of cortisone on production of granulation tissue in the rabbit. Proc. Soc. Exp. Biol. and Med., *72:* 718–721.

PLATE 1

EXPLANATION OF FIGURES

1 Cheek pouch of control hamster. Artery and vein. In vivo, transmitted light. × 50.

2 Same view as 1 taken one minute after intraperitoneal injection of epinephrine. Note the marked constriction of the artery. × 50.

3 Cheek pouch of cortisone-treated hamster. Artery and vein. In vivo, transmitted light. × 50.

4 Same view as 3 taken 5 minutes after intraperitoneal injection of epinephrine. Note the slight degree of contraction of the artery. × 50.

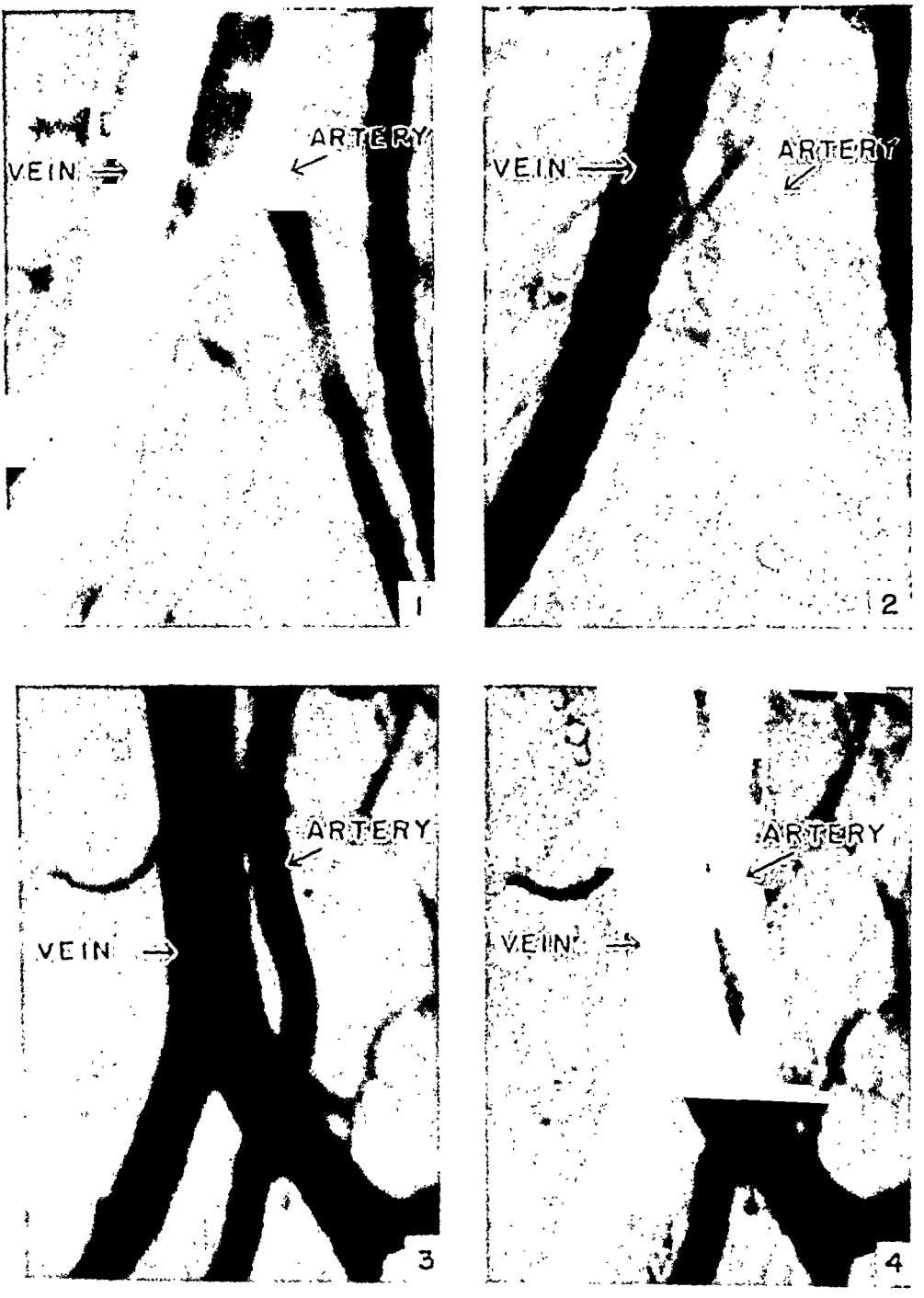
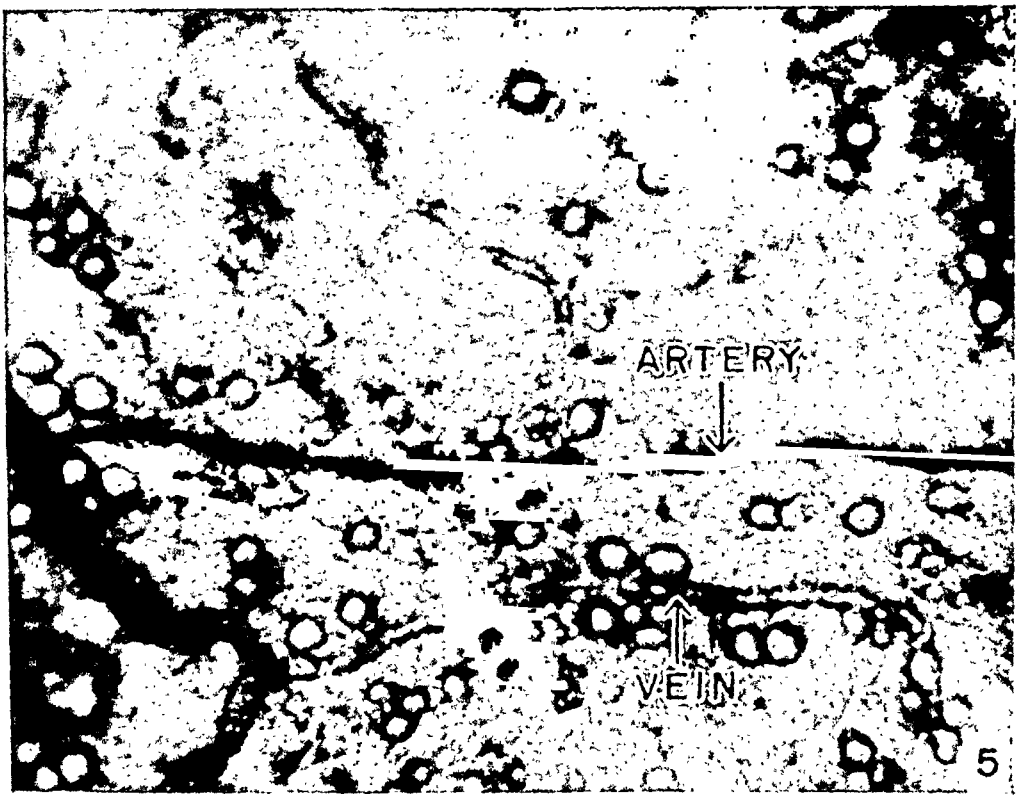

VEIN
ARTERY
1
VEIN
ARTERY
2
ARTERY
VEIN
3
ARTERY
VEIN
4

PLATE 2

EXPLANATION OF FIGURES

5 Control hamster cheek pouch tissue. In vitro, transmitted light. × 30.

6 Same view as 5 taken one minute after adding epinephrine to Ringer's fluid bathing the excised piece of tissue. Note that the arterial contraction had forced the blood cells from the lumen. × 30.


ARTERY
VEIN
5

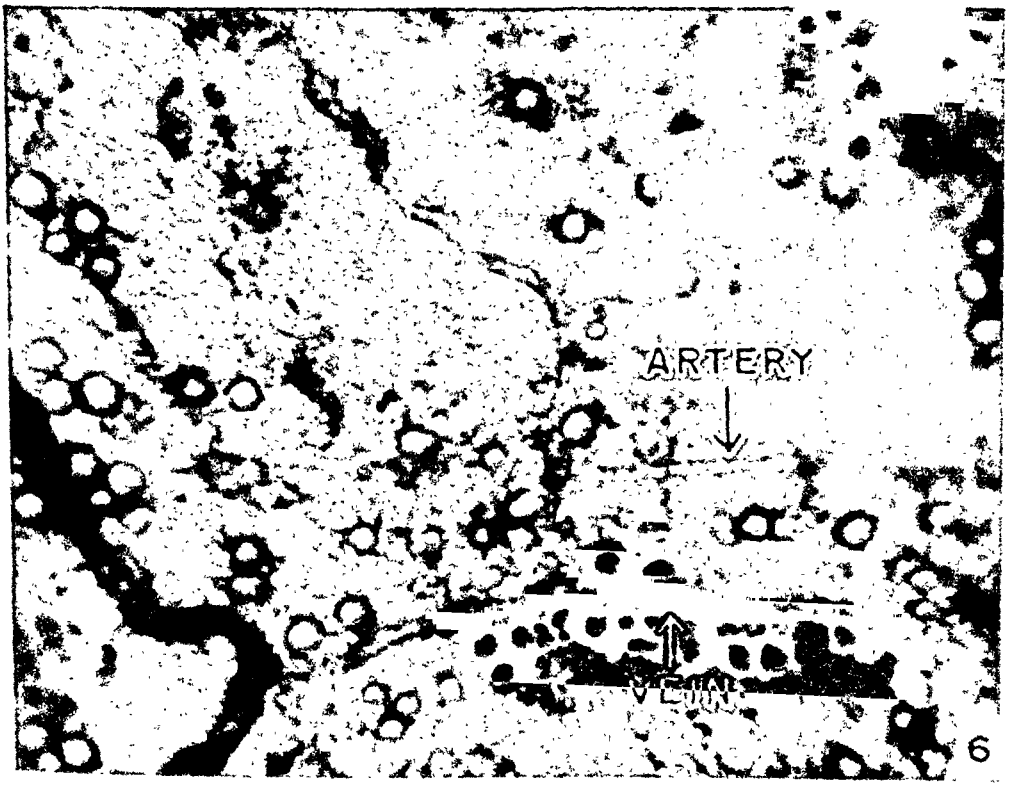
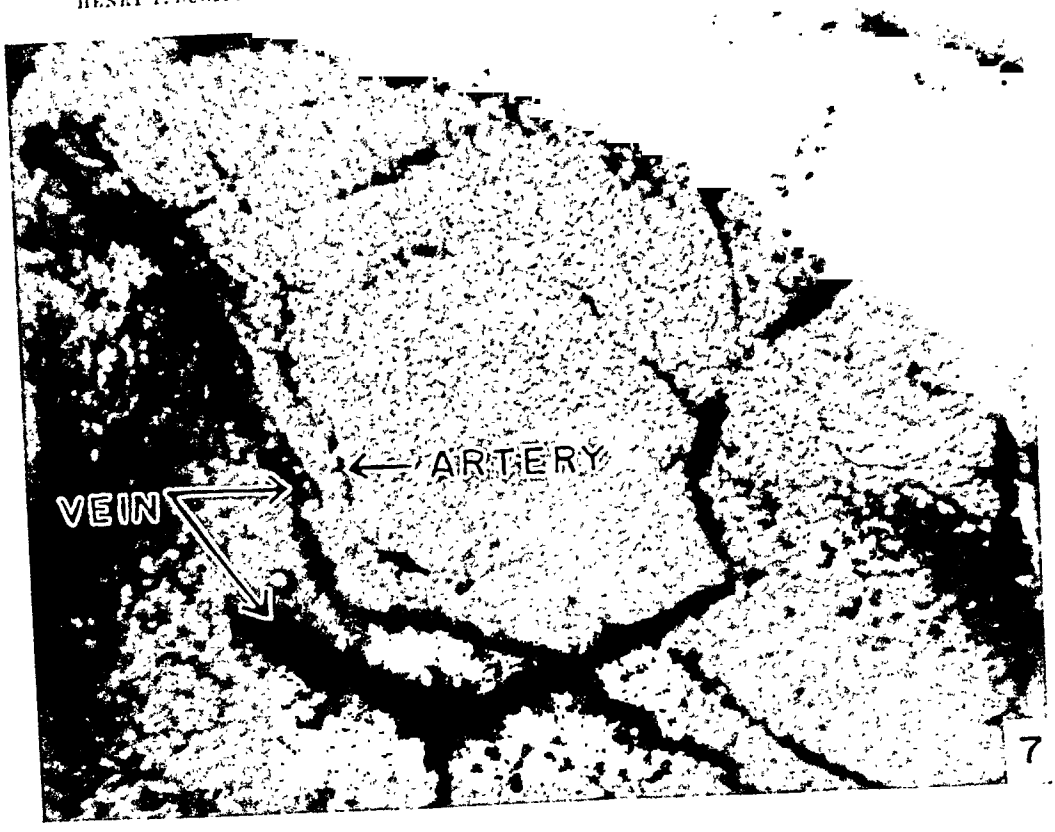

ARTERY
VEIN
6

PLATE 3

EXPLANATION OF FIGURES

7 Cortisone-treated hamster cheek pouch. In vitro, transmitted light. × 30.

8 Same view as 7 taken 5 minutes after epinephrine addition. Note that the artery has constricted only slightly and blood is still present in the lumen. × 30.


VEIN
ARTERY
7

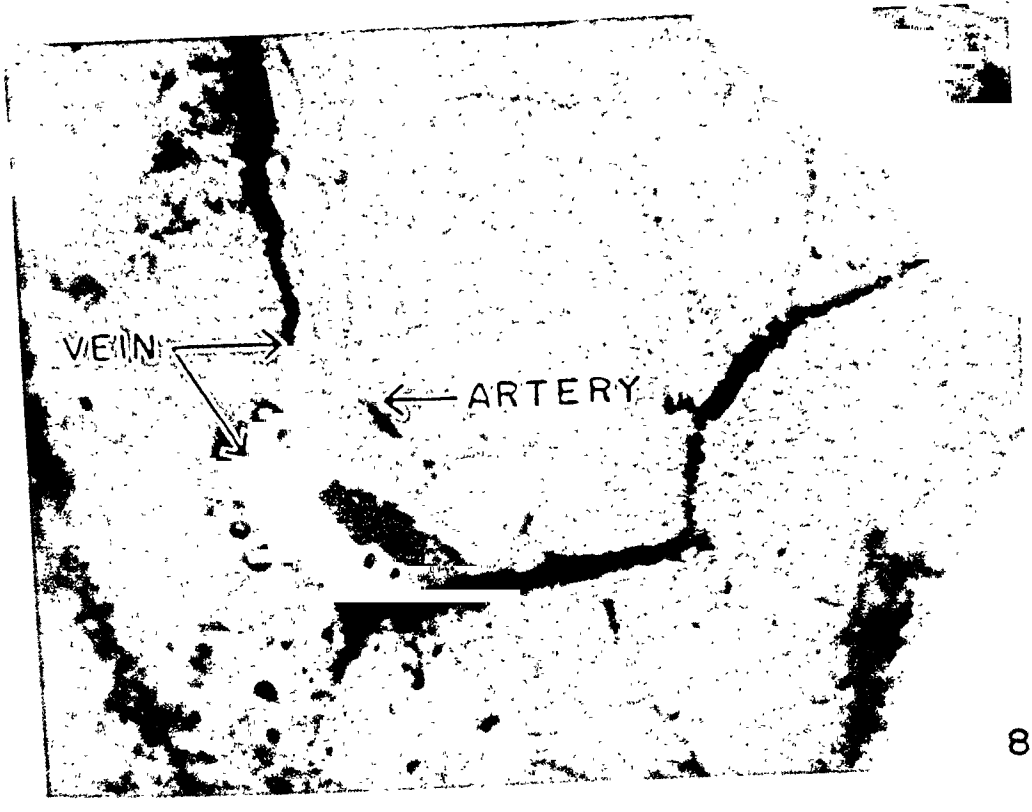
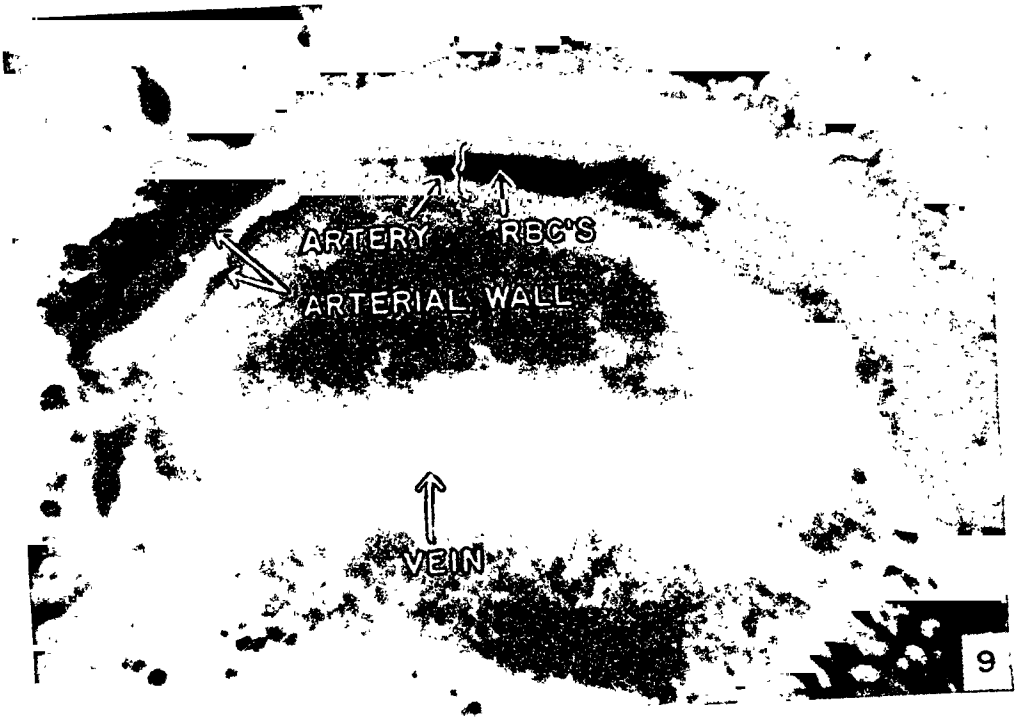

VEIN
ARTERY
8

PLATE 4

EXPLANATION OF FIGURES

9 Femoral artery and vein from a control hamster. In vitro, transmitted light. × 8.

10 Same view as 9 taken immediately after addition of epinephrine. Note the beginning arterial constrictions at regions marked "X." × 8.


ARTERY
RBC'S
ARTERIAL WALL
VEIN
9

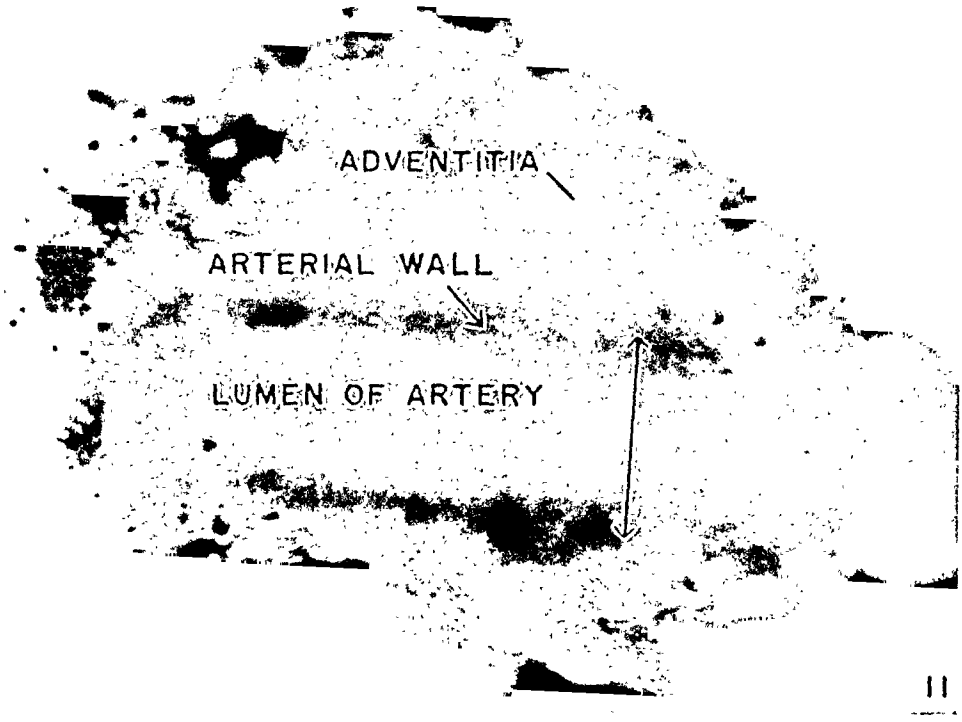

11 X 11
10

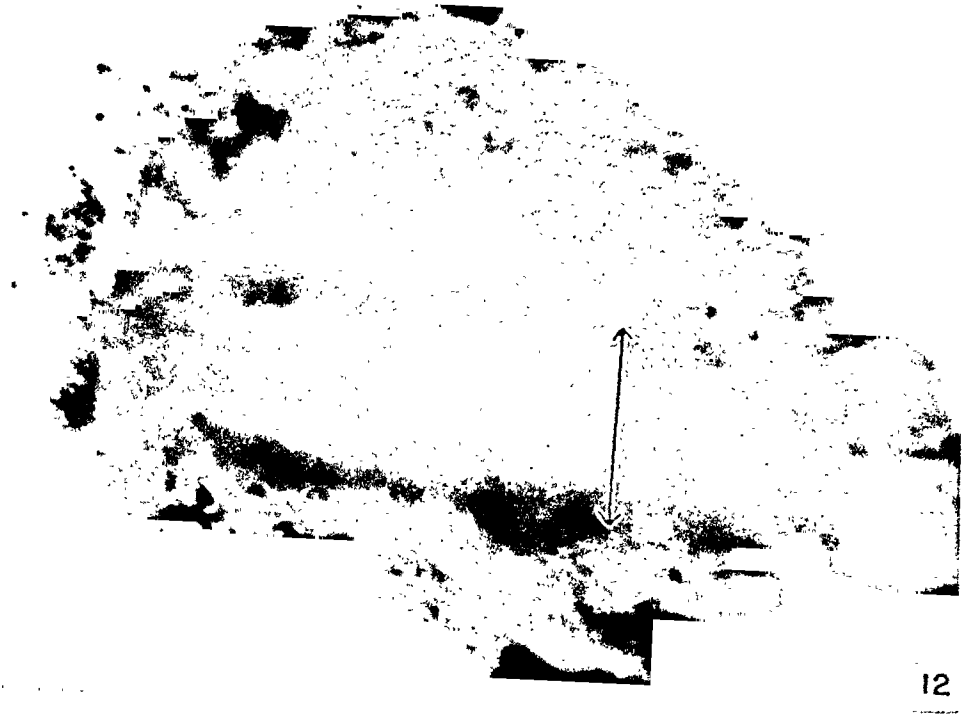
PLATE 5

EXPLANATION OF FIGURES

11 Femoral artery from a cortisone-treated hamster. In vitro, transmitted light. × 50.

12 Same view as 11 taken 5 minutes after adding epinephrine. Only a slight reduction in vessel diameter is discernible. × 50.


ADVENTITIA
ARTERIAL WALL
LUMEN OF ARTERY
11


12

FORMATION OF GIANT CELLS FROM HUMAN MONOCYTES CULTIVATED ON CELLOPHANE

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SIX FIGURES

The origin, mode of formation, and significance of foreign body giant cells has been investigated by many workers in the fields of pathology and microscopic anatomy. After Langhans' (1868) classic description of the giant cells in tuberculosis, many papers appeared in the scientific literature on this subject. Many workers introduced substances and live organisms into experimental animals in attempts to induce the formation of giant cells (Haythorn, '27; Hektoen, 1898; Sabin and Doan, '27; Sabin, Doan and Forkner, '30). From the multitude of articles published during the period of 1870 to 1930, it is obvious that there was little agreement about the source of these cells, the method of their formation, or their functional importance.

The experimental production of giant cells in vitro added much evidence to support the theory of their formation from mononuclear leucocytes (Lewis, '25; Lewis and Lewis, '25; Lewis and Lewis, '26; Lewis, '27; Maximow, '24). Awrorow and Timofejewsky ('14) were the first to demonstrate that giant cells could be formed from circulating leucocytes. Later Timofejewsky and Benewolenskaja ('25, '27, '27a, '28) produced giant cells in cultures of human and rabbit leucocytes inoculated with tubercle bacilli. They, like many other in-

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vestigators who have employed tissue culture methods, failed to mention the exact mode of formation of giant cells and the time needed for their development. This was undoubtedly due to the difficulty of producing these cells with regularity, especially with human leucocytes.

This paper describes a simple, reproducible method for forming giant cells from human leucocytes cultivated in vitro, and provides a means of observing in great detail the stages in their formation. The method of tissue culture also offers an excellent opportunity to study the process of phagocystosis and intracellular digestion.

MATERIAL AND METHODS

Cultures were made from the buffy coat. Fifteen cubic centimeters of human blood were obtained from a cubital vein and placed immediately in a 36 cm^3 round pyrex bottle which contained 1.5 cm^3 of a 0.172 molar (3.0%) solution of sodium citrate. The blood was then pipetted into 13 mm $\times$ 100 mm test tubes which were housed in 22 mm $\times$ 150 mm screw capped vials and centrifuged at 1700 R.P.M. for 10 minutes. The supernatant plasma was removed with a dropper pipette. The excess plasma was washed away by adding a 0.152 molar (0.9%) solution of sodium chloride to the test tube, allowing it to run down the sides, and then carefully removing it with a dropper pipette. This procedure was done twice and a solution of 0.00275 molar calcium chloride (11 mg Ca/100 cm^3) in 0.9% sodium chloride was added and allowed to remain over the buffy coat for 10 minutes. The clotted buffy coat was then firm enough to loosen with a No. 10 Bard Parker blade attached to a No. 7 Bard Parker scapel. It was carefully placed in a small, black embryological watch glass which had been set in a 100 mm $\times$ 22 mm petri dish. This procedure prevented the cells from drying. The disc was washed in two changes of Gey's physiological salt solution and cut into approximately 1-mm^3 pieces. Three pieces were explanted on the surface of perforated cellophane (Earle, Evans and

Schilling, '50; Evans and Earle, '47) which had been placed in the D-5 Carrel flasks.[3] One and eight-tenths cubic centimeters of pooled human serum diluted with Gey's solution in a ratio of two parts Gey's solution with one part serum was added. The flasks were then sealed with rubber stoppers and incubated at 37°C. It took about one hour to prepare a series of cultures in 12 to 18 Carrel flasks.

After explantation, one to two hours usually elapsed before there was a good area of migration. Two hours after incubation was therefore designated as the zero fixation period. Often during later periods of cultivation, the leucocyte disc would become loose and settle on another part of the cellophane. In this way there were often three to 4 more areas which contained many leucocytes.

The cellophane cultures were fixed at various intervals of time. Before fixation, the medium was removed from the flasks and the cultures washed with Gey's solution. The cultures were fixed for 20 minutes in either Carnoy's solution, Zenker-formol or 2% osmium tetroxide vapor. They were then washed for one hour in tap water, rinsed thoroughly in distilled water, and stained. Some Zenker-formol fixed tissues were stained with Hematoxylin eosin azure II. Others fixed in Zenker-formol or Carnoy's were stained by a modification of the Feulgen technique (Stowell, '45) and counterstained with fast green. Some of the cultures fixed in osmium tetroxide vapor were lightly counterstained with eosin.

OBSERVATIONS

Four hundred and fourteen explants in all were studied. Usually there were few leucocytes on the surface of the cellophane before two hours of incubation, but after this period, there was a dense migration from the explants. Cultures fixed at this stage also contained many cells in the area below

[3] The cellophane, Dupont, pt. 62, thickness 450, with perforation design 08 can be obtained from Microbiological Associates, P. O. Box 5970, Washington 14, D. C. This design has 325 perforations each of 0.023-inch diameter, to the square inch and 13.7 open area.

the explanted piece of disc. The polymorphonuclear leucocytes migrated the fastest and many were found some distance from the original explant. Monocytes and lymphocytes were dispersed among the granular leucocytes; in some areas there were large concentrations of these cells, but usually at this early period of incubation they were evenly dispersed.

The granular and non-granular leucocytes were easy to distinguish in both living cultures and in fixed and stained preparations. In fixed cultures of two hours' incubation some polymorphonuclear leucocytes contained a few vacuoles; the chromatin strands of many granular leucocytes were absent, and there was an increase in the number of lobes which varied in shape and size. Five to 6 lobes were found in a single granular leucocyte and sometimes these irregularly shaped lobes looked as though they were being extruded from the cell, a phenomenon which became more evident as the cultures were incubated for longer periods of time. At this early period of incubation, debris had already been phagocytosed by the monocytes (fig. 1). The monocytes had very irregular nuclei and in living cultures the cells could be seen sending out pseudopodia; some had already formed small aggregates. A few monocytes had fused with each other by three hours of incubation, and the cytoplasmic area had increased.

The mononuclear cells were ameboid and actively phagocytosed debris. Their eccentrically located nuclei varied considerably in shape and the hypertrophied cytoplasm had an irregular outline. Many of these cells continued to aggregate during the first 24 hours. Bizarre, tongue-like projections sometimes extended from these nuclei. Some nuclei of the mononuclear cells appeared to be undergoing amitosis, but one was not able to follow them in vitro to be certain that they actually did divide. However, fixed cultures gave the impression that some of these nuclei had divided in this manner. One was impressed by the elasticity of the nuclei and their ability to move or be moved about in the cytoplasm. This phenomenon was very evident in older cultures, espe-

cially where nuclei could be seen in the process of passing from one giant cell to another (fig. 4).

During the first 24 hours of incubation, the lymphocytes appeared inactive; some were pycnotic and were being phagocytosed by the mononuclears. At the end of this period the detritus of many polymorphonuclear leucocytes was seen on the cellophane and those that were still intact contained large vacuoles. Some of these granular leucocytes contained nuclei with as many as 12 irregularly shaped lobes between which the chromatin threads were usually missing. Many mononuclear cells were in the process of fusing, which led to the formation of the large multinucleated cells. In fixed cultures of 20 to 24 hours, the more isolated mononuclear cells were seen to have enlarged; their nuclei were eccentrically located. Many small giant cells had already formed by 24 hours of cultivation, and many of the mononuclear cells could be seen being drawn into the giant cells.

At 40 hours of incubation the multinucleated cells had increased in size. A cell membrane was difficult to see in living cells, but in fixed cultures a distinct membrane was seen both in the mononucleated epithelioid and giant cells. The central areas of the giant cells with peripherally arranged nuclei stained intensely, and in those giant cells where the nuclei were scattered throughout the cytoplasm, this intense staining reaction was noted in the areas surrounding the nuclei. The area surrounding the nucleus in the epithelioid cell stained in a similar manner. If many giant cells were close to each other, then the lightly staining peripheral cytoplasm was not observed (fig. 5), but it appeared in cultures where giant cells were some distance apart (fig. 3). This is common in giant cells produced in vivo. Fibrous-like condensations in the cytoplasm were seen in many giant and epithelioid cells. These strands which varied in length, stained more intensely than the rest of the cytoplasm and were more numerous in older cultures (fig. 4). In those giant cells which contained peripherally located nuclei, the phagocytosed material was concentrated in the central area, while in those cells where

the nuclei were scattered throughout the cytoplasm the phagocytosed material was distributed throughout the cell.

In cultures of 50 to 60 hours of incubation many small refractory globules appeared in the peripheral cytoplasm in living cultures, and in stained preparations they were seen as small vacuoles. These vacuoles increased greatly in number in older cultures so that many of them were seen in the protoplasm which extended from the giant cells. Fusion of giant cells and of many epithelioid cells with giant cells occurred during this period of cultivation. This phenomenon increased with longer cultivation. Bridges formed between giant cells by the extension of the cytoplasm of one cell and its fusion with that of another giant cell; nuclei would then pass from one giant cell to another. In this manner giant cells in older cultures increased tremendously in size and in the number of nuclei which they contained.

Protoplasmic bridges formed between cells that were some distance apart. It appeared that the strands in the cytoplasm mentioned above acted as a framework for the formation of the connecting bridges between giant cells; the nuclei then passed from cell to cell through these cylinders. In giant cells that lay close to one another the cell membrane between the cells disappeared and there formed a continuous large multinucleated mass (fig. 6). Polymorphonuclear leucocytes were no longer seen on the cellophane after 50 hours of incubation, but a few were found within giant cells in the process of being digested.

After 60 to 75 hours of incubation it became more evident that the intensity of the staining reaction in the cytoplasm was related to the position of the nuclei. In general the nuclei of these older cultures were round or oval, but some cells contained many irregularly shaped nuclei. Some of these nuclei appeared to be undergoing amitosis.

In cultures incubated 80 to 90 hours, a few mitotic figures were present in the peripheral areas of the giant cells, but this was rare. It has also been seen very infrequently in cultures of rabbit leucocytes (Goldstein, unpublished data).

After 90 to 100 hours of incubation the cell membranes appeared coarse and irregular in living cultures. The nuclei also stood out more clearly against the cytoplasm.

The cultures were observed up to 170 hours. After this time most of the cells had broken down and very few could be seen adhering to the cellophane. There was an increased tendency for clumping of the nuclear material and an enlargement of vacuoles. Though the giant cells appeared to be degenerating during these later stages, many cells were still being phagocytosed by them; this occurred during the entire culture period in the more densely populated areas of migration.

DISCUSSION

The cellophane was the principal factor in the rapid formation of the giant cells. In order to exclude other factors, such as the sodium citrate and calcium chloride, pieces of buffy coat were explanted in plasma clot cultures in D-5 Carrel flasks. The disc was prepared in the same manner as it was for the explantation on cellophane. The pieces of buffy coat were explanted in human plasma which was diluted with Gey's solution to approximate the concentration of serum used when explants were made on cellophane. Although there was a dense migration of mononuclear leucocytes into the plasma, there was no indication of giant cell formation. The mononuclear cells became spindle shaped after a few days. Frequently in these cultures the plasma would liquefy and the entire clot was lost. In those plasma clot cultures in which liquefaction did not rapidly occur, giant cells developed in the region below the explanted disc. This phenomenon could not be seen until 5 to 6 days after preparation of the culture. At this time the disc would become loose and break away from the surface of the plasma. The giant cells were seen adhering to the glass surface of the Carrel flask. In this case, the glass surface acted as a stimulant for the formation of giant cells, which were few in number.

Although other investigators have reported that amitosis was the principal mode of formation of giant cells (Awrorow

and Timofejewsky, '14; Timofejewsky and Benewolenskaja, '27, '28), this was not the case in these cultures explanted on cellophane. The giant cells formed by fusion of mononuclear cells derived from the monocytes. There was some evidence of amitosis; however, one could not follow the devision of the nuclei. There was a difference in the size of the nuclei in some giant cells and in some cells nuclear constriction could be seen. These observations in fixed and stained cultures suggested that amitosis may have occurred. Mitotic figures were rarely seen in these cultures.

The so-called "Epithelioid cell" appeared at the same time as did the giant cell; it was a hypertrophied derivative of the monocyte and could also fuse to form multinucleated giant cells. Since it appeared simultaneously with the giant cells, it seemed possible that the epithelioid cell was a mononucleated giant cell; this observation was confirmed in older cultures. Many experimenters, however, still consider the epithelioid cell to be a stage in the formation of the foreign body giant cell.

Since foreign body giant cells with nuclei arranged around the periphery and those with nuclei scattered throughout the cytoplasm were seen in the same cultures, and since both arose from monocytes, it was concluded that there was no real difference between the two types; this conclusion disagrees with the views of Sabin ('23, '30), Forkner ('30), and others (Cunningham, Sabin and Doan, '25, '25a; Haythorn, '27). It was observed that the arrangement of the nuclei depended largely on when the mononuclear cells fused and the number of cells present at the time of fusion. The arrangement of the nuclei was, therefore, a matter of chance.

If the monocytes were of a polyblast nature, there was no indication of this in the culture of the buffy coat on cellophane. All of these cells developed into foreign body giant cells. Hektoen (1898) claimed that he was able to observe the migration of cellular elements from the giant cells in experiments in vivo, and Cohen ('26) stated that a similar phenomenon occurred in his cultures of turtle blood. These

changes were not noted in the culture of the human buffy coat on cellophane. Instead there was a tendency for the fusion not only of mononuclear cells, but also of the large multinucleated giant cells (fig. 6).

SUMMARY

A simple method for the rapid production of foreign body giant cells from human leucocytes is described. The procedure involves the cultivation of the buffy coat from human peripheral blood on the surface of perforated cellophane in D-5 Carrel flasks. The monocytes were the cells that gave rise to the foreign body giant cells. It is possible to follow the stages in the formation of giant cells in great detail both in living cultures and in fixed and stained preparations. Giant cells appeared in all cultures incubated for from 20 hours to 7 days in vitro.

ACKNOWLEDGMENT

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LITERATURE CITED

AWROROW, P. P., AND A. TIMOFEJEWSKY 1914 Kultivierungsversuche von Leukämischem Blute. Virch. Arch. f. Path. Anat. u. Physiol., *216:* 184–214.

COHEN, M. 1926 Observations on the formation of giant cells in turtle blood. Am. J. Path., *2:* 431–436.

CUNNINGHAM, R. S., F. R. SABIN AND C. A. DOAN 1925 The role of the monocyte in tuberculosis. Bull. Johns Hopkins Hosp., *37:* 231–280.

——— 1925a The development of leucocytes, lymphocytes and monocytes from a specific stem cell in adult tissue. Cont. to Emb. Carn. Inst. Wash., *16:* 229–273.

EARLE, W. R., V. J. EVANS, M. F. EDWARD AND E. DRECHSLER 1949 Influence of perforation design on growth of tissue cells under perforated cellophane sheets in vitro. J. Nat. Cancer Inst., *10:* 291–295.

EARLE, W. R., V. J. EVANS AND E. L. SCHILLING 1950 Extension of cellophane substrate procedure to growth of in vitro cultures of large area. J. Nat. Cancer Inst., *10:* 943–967.

EVANS, V. J., AND W. R. EARLE 1947 The use of perforated cellophane for the growth of cells in tissue culture. J. Nat. Cancer Inst., *8:* 103–119.

FORKNER, C. E. 1930 The origin and fate of two types of multinucleated giant cells in circulatory blood. J. Exp. Med., *52:* 279–295.

HAYTHORN, S. R. 1927 Multinucleated giant cells with particular reference to the foreign body cells. Arch. Path., *7:* 651–713.

HEKTOEN, L. 1898 The fate of giant cells which form in the absorption of coagulated blood serum in the anterior chamber of the rabbit's eye. J. Exp. Med., *3:* 573–578.

LAMBERT, R. A. 1912 The production of foreign body giant cells in vitro. J. Exp. Med., *15:* 510–515.

LANGHANS, T. 1868 Über Riesenzellen mit Wanständzer Kernen in Tuberkieln und die Fibrose Form des Tuberkels. Virch. Arch. f. Path. Anat., *42:* 382–404.

LEWIS, M. R. 1925 The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood. Am. J. Path., *1:* 91–99.

LEWIS, M. R., AND W. H. LEWIS 1926 Transformation of mononuclear blood cells into macrophages, epithelioid, and giant cells in hanging drop cultures from lower vertebrates. Cont. to Emb. Carn. Inst. Wash., *18:* 97–120.

LEWIS, W. H., AND M. R. LEWIS 1925 Transformation of white blood cells. Jour. A.M.A., *84:* 798–799.

LEWIS, W. H. 1927 The formation of giant cells in tissue culture and their similarity to those in tubercular lesions. Am. Rev. Tuberc., *15:* 616–628.

MAXIMOW, A. 1924 Tuberculosis of mammalian tissue in vitro. J. Infect. Dis., *34:* 549–584.

———— 1925 Role of non-granular leucocytes in the formation of the tubercle. J. Infect. Dis., *37:* 418–429.

SABIN, F. R. 1923 Studies of human living blood cells. Bull. Johns Hopkins Hosp., *34:* 277–288.

SABIN, F. R., AND C. A. DOAN 1927 The relation of monocytes and clasmatocytes to early infection in rabbits with tubercle bacilli. J. Exp. Med., *46:* 621–644.

SABIN, F. R., C. A. DOAN AND C. E. FORKNER 1930 Studies on tuberculosis. J. Exp. Med., supplement no. 3, *52:* 1–96.

STOWELL, R. E. 1945 Feulgen reaction for thymonucleic acid. Stain Tech., *20:* 45–58.

TIMOFEJEWSKY, A. 1928 Über Leukozytenkulturen des Menschenblutes in Vitro. Arch. f. Exper. Zellforsch., *6:* 259–264.

TIMOFEJEWSKY, A., AND S. BENEWOLENSKAJA 1925 Explantationsversuche von Weissen Blutkörperchen mit Tuberkelbazillen. Arch. f. Exper. Zellforsch., *2:* 31–42.

———— 1927 Tuberculous inoculation in cultures of leucocytes of the human blood. Arch. f. Exper. Zellforsch., *4:* 64–78.

———— 1927a Zur Frage Über die Reaktion Pathologische Leukozytenformen des Menschenblutes in Vitro auf Tuberkel Bazillen. Virch. Arch. f. Path. Anat. u. Physiol., *264:* 605–617.

———— 1928 Über Tuberkulose Infektion von Gewebskulturen. Arch. f. Exper. Zellforsch., *6:* 230–234.

PLATES

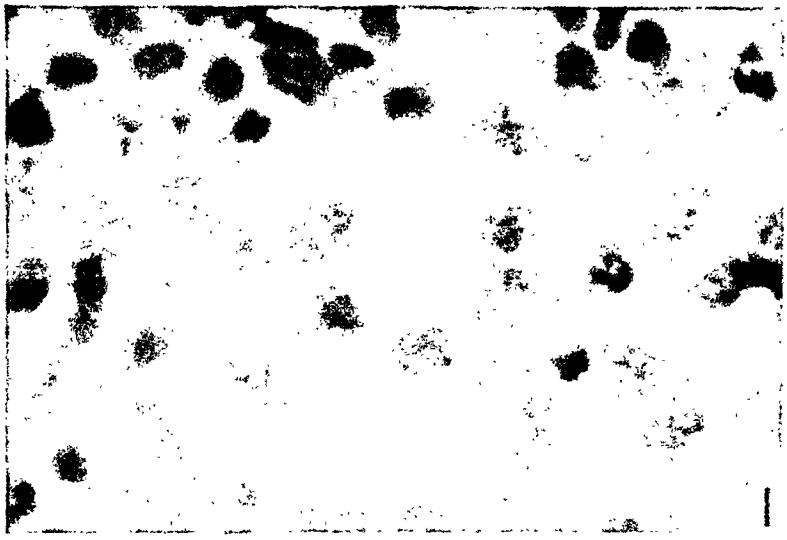
PLATE 1

EXPLANATION OF FIGURES

1 Phagocytosis of debris by mononuclears on cellophane. Note particles in cell cytoplasm and the varied shapes of the nuclei. Incubated two hours. Zenker-formol. Hematoxylin eosin azure II. × 1000.

2 Beginning of fusion of a group of mononuclears on surface of cellophane. Incubated 21 hours. Zenker-formol. Hematoxylin eosin azure II. × 1000.

3 Note the fusion of large mononuclear cells with the giant cell. There are many cytoplasmic strands in the giant cell. Incubated 74 hours. Zenker-formol. Hematoxylin eosin azure II. × 450.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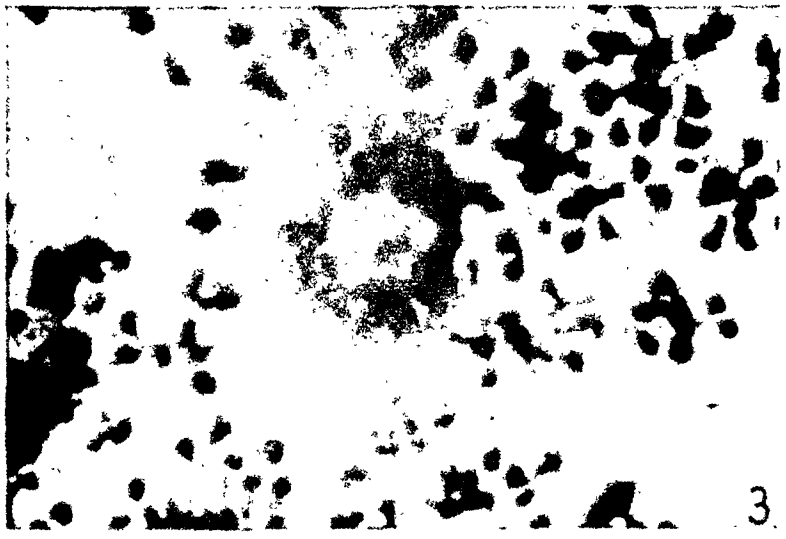

3

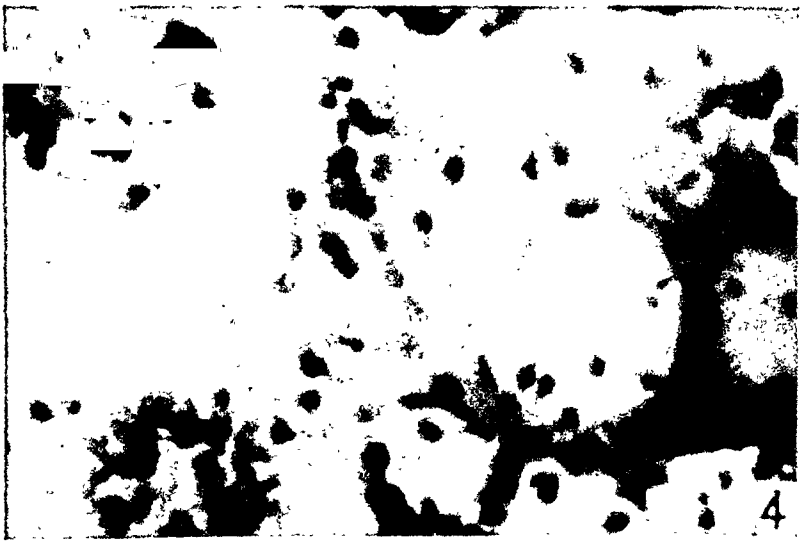
PLATE 2

EXPLANATION OF FIGURES

4 Many bridges of cytoplasm connect the giant cells. The nuclei pass through these bridges into the larger cells. There are also many cytoplasmic strands in these cells. Incubated 74 hours. Zenker-formol. Hematoxylin eosin azure II. × 450.

5 Note the sheet-like arrangement of many closely packed giant cells. Incubated 83 hours. Carnoy. Feulgen and fast green. × 100.

6 Three large multinucleated giant cells are in the process of fusing. Incubated 92½ hours. Zenker-formol. Hematoxylin eosin azure II. × 450.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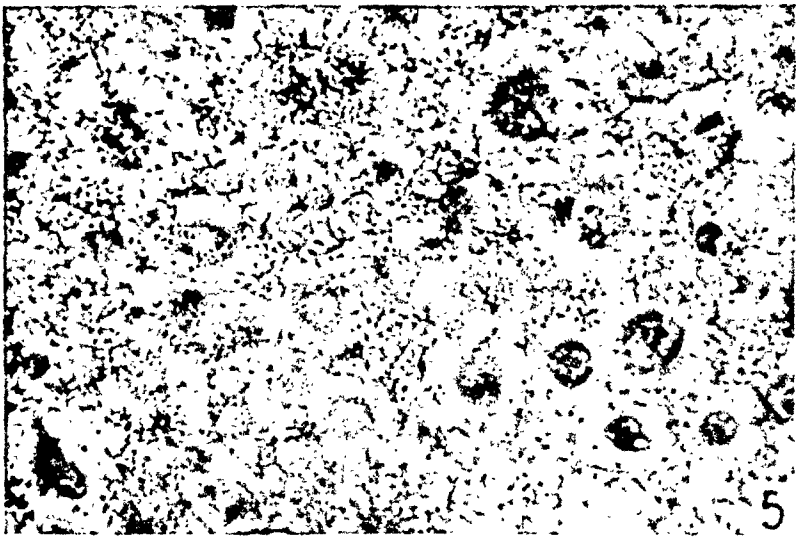
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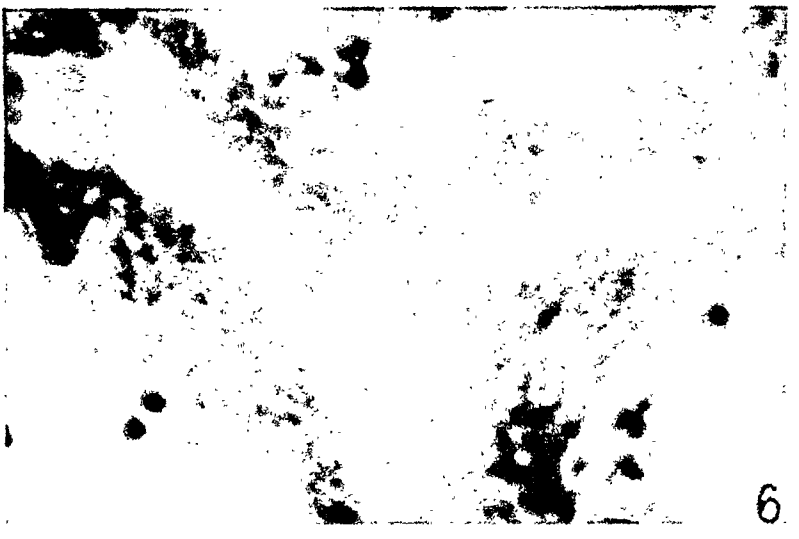
6

CYTOLOGICAL CHANGES IN THE RAT ANTERIOR PITUITARY FROM BIRTH TO MATURITY[1]

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TWENTY-FOUR FIGURES

Two valuable staining procedures are now available for the study of cell types in the rat anterior pituitary: the McManus periodic acid-Schiff (PAS) technique, first applied to the pituitary by Catchpole ('47), and Gomori's aldehyde-fuchsin stain, which has been extensively employed by Halmi ('50, '51, and '52). With the aid of these techniques, Purves and Griesbach, continuing and amplifying the concept of Romeis ('40) and Halmi ('50), distinguished two types of basophils, the thyrotrophs and the gonadotrophs, primarily on the basis of their response to hormone treatments ('51a, '51b). In the opinion of Purves and Griesbach, these basophils differ not only in their morphology and location in the gland of the adult rat but also in their functions. If this opinion is correct, 5 of the pituitary hormones could be linked to special types of cells in the anterior pituitary of the rat. The three glycoprotein-containing hormones could be connected to the PAS-positive "basophils," of which the "gonadotrophs" would produce the follicle-stimulating (FSH) and the luteinizing (LH) hormones, while the "thyrotrophs" would be responsible for the thyroid-stimulating (TSH) hormone. The acidophils, of which there might be two groups (from evidence in other species), would be the site for the

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[2] Edith Claypole Memorial Fellow.

production of growth hormone and luteotrophic (lactogenic) hormone. The source of the adrenocorticotrophic hormone is not established.

In the present report we have studied the development of the acidophils and of the thyrotrophin-producing and gonadotrophin-producing basophils in the growing rat from birth to 11 weeks of age.

METHODS

The pituitaries of 229 rats of the Sprague-Dawley strain and 40 rats of the Otago Wistar strain were studied. The young were allowed to remain with their mothers until the 4th week. Thereafter they were fed on Purina laboratory chow. Rats were sacrificed at various ages from the day of birth, designated as day one, through day 77. In order to insure even sampling, only one male and one female from a single litter were included in each age group. It was useful to fix the pituitaries of the younger animals (age up to 14 days) *in situ* and remove the glands from the skull after 60 minutes of fixation. Sublimate-formalin was used as fixative, and paraffin embedding was employed after dehydration. The entire gland was cut in the horizontal plane at 3 and 5 μ. The 5-μ sections were stained by the periodic acid-Schiff technique after the manner of Purves and Griesbach ('51a) and by Gomori's aldehyde-fuchsin stain (Gomori, '50). The 3-μ sections were stained by Crossmon's modification of Mallory's triple stain (Crossmon, '37).

The staining power of different batches of aldehyde-fuchsin varied greatly. Some batches failed to stain the basophils, while other batches were effective for as long as 6 months. The stain was always tested on sections known to contain Gomori-positive cells just before being used for experimental slices. When Gomori staining was successful, sections were generally counterstained by one of three methods: (1) a type of Mallory triple stain with fast green substituted for aniline blue (Halmi, '50); (2) a one-step orange G-light green stain (Halmi, '52); (3) Heidenhain's Azan with fast green (Gomori,

'50). If the pituitaries had been immersed in iodine-alcohol before dehydration, a dark and not specifically stained marginal zone was observed after aldehyde-fuchsin staining. It was found desirable to treat sections with iodine-alcohol only after removing the paraffin in the usual way.

The term "sex zone" used in the text refers to the region in the pituitary where large numbers of gonadotrophs were found in a small area (Purves and Griesbach, '51a, fig. 1-B).

RESULTS

Sections of anterior pituitaries from the first week of life gave the impression, under low-power examination, of being composed almost entirely of numerous small nuclei closely packed together. Small cell size and, consequently, close aggregation of nuclei were, in fact, striking characteristics of pituitaries up to day 28. Full development in cell size and morphology was not observed until approximately day 56.

Mitoses in all cell types were apparent at quite early stages. Between days 14 and 28, the mitotic activity was increased, the maximum being observed in both sexes during the 4th week. Smaller numbers of mitoses were seen in glands as old as day 42.

The history of the postnatal development of the cell types in Sprague-Dawley rat pituitaries has been organized here into a series of periods or stages as follows: (1) the first week of life, when chromophobes constitute the great majority of the pituitary cells and chromophils are difficult to distinguish due to their minute size and faint staining ability; (2) the second week, when chromophils become more numerous, increase in size, and stain brighter; (3) the third and 4th weeks, when basophils are approximately equal in number to acidophils and appear to aggregate in special areas; (4) the 5th to 7th weeks, when differences between the sexes are established. The female gonadotrophs have undergone degranulation, large numbers of typical granulated gonadotrophs are present in the male, and thyrotrophs in both sexes

are plentiful and well granulated; (5) the 8th week and onwards, when pituitaries present an essentially mature appearance.

Week one

On the first day of life, the majority of cells in any section are "chromophobes." The presence of chromophil cells could be determined only under high magnification (oil immersion), as the cells are quite small and the staining reaction faint. Sections stained by Crossmon's method revealed no acidophils in 5 cases; the other pituitaries showed only small numbers of acidophils in each section. In all positive cases, the cytoplasm of acidophils appeared as an extremely thin, faintly red or orange-staining ring adhering to the nuclear wall. The nuclei were clear and vesicular, and contained fine chromatin particles.

Crossmon's method, even in 5 μ sections of newborn pituitaries, was not so effective as the PAS technique in exposing basophils. Blue-stained basophils were not found during the first week, but purplish, large cells were visible. After application of PAS reagent, all pituitaries contained 10–20 or more cells, per section, which showed varying degrees of pink staining, indicating the presence of glycoprotein. These cells were notably larger than the acidophils, and had a round or oval shape. Cytoplasmic details were not discernible, other than occasional, extremely fine granules. Nuclei were similar to those of the acidophils. The PAS-positive cells seemed to be in close contact with the reticular tissue of the sinusoids. Mitoses were generally frequent (figs. 1 and 3); they could occasionally be found in the pink "basophils," but most of the dividing cells were colorless.

In sections stained with Gomori's aldehyde-fuchsin stain, small cells containing a rim of fine purple granules around the nucleus were seen along the connective tissue septa in the central part of the anterior lobe (fig. 4). These cells generally occurred in strings or small groups, and were fewer in number than the total PAS-staining cells from the same

gland. At this stage the reticulum of the sinusoids was positive to the Gomori stain.

From days three to 7, all cell types increased in numbers, and their characteristic features were generally the same as described for day one. But on day 7, some distinct PAS-positive granules could be seen in basophil cells.

Weeks two to four

Day 9 and day 11. The glands of the 9- and 11-day-old rats resembled closely those found on the 7th day.

Day 14. A more pronounced change in the pituitary histology occurred between days 11 and 14. On day 14 the pituitaries showed many chromophils, and the distinction between chromophobes and chromophils was much clearer than during the first 11 days because of increased cell size and tinctorial reactions.

In the female pituitaries, basophils (aniline blue and PAS-positive cells) were obviously concentrated in the area adjacent to the intermediate lobe and especially at the anterior edge in the so-called "sex zone." There was thus a definite regional predilection evident here for the first time. While male pituitaries had not yet advanced to exactly the same stage at 14 days, they also showed cells with stronger PAS staining in the "sex zone" along blood vessels, but these were not so copious as in the female. In both sexes some of the "basophils," both in the "sex zone" and in the central region, had a shrunken appearance and seemed to consist of a ring of cytoplasm separated from the nucleus by a thin, unstained space. The nucleus of such a cell was pyknotic, the cytoplasm in the "sex zone" cells stained intensely with aniline blue or PAS, while in the central gonadotrophs, the cytoplasmic ring consisted of sparse, light pink flocks.

Basophils were also quite numerous in the central area of the anterior lobe, although not so concentrated there, in either sex, as along the intermediate lobe and in the "sex zone."

Their morphology at this stage appeared similar to that found in the sex region. However, not all of the central "basophils" were round — some were angular in shape. These were positive to aldehyde-fuchsin staining, showing granules outlining the cell walls and the nuclei, and not yet filling completely the interior of the cells (figs. 6–8).

One exceptional gland was noted in this group; it showed few basophils as judged by all three methods of staining.

The acidophils of the 14-day-old rats, though far more developed than during the first week, were nevertheless present in smaller proportions than in the adult glands. Sections of 14-day pituitaries stained by Crossmon's method appeared to contain basophils and acidophils in equal proportions, and this was confirmed by cell counts of two of the glands. In young pituitaries, all chromophils were located in the immediate vicinity of blood vessels. They seemed to be growing along the sinusoids, often palisade-like, or in clusters.

Day 21. In general, the descriptions of the 14-day-old rat pituitaries may be applied here. Acidophils were more numerous than at day 14, but still far below the proportions found in the adult rat. They had acquired a greater tendency to stain with acid fuchsin. The basophils were also more conspicuous, having become larger, darker-staining and more numerous. The sex zones in both sexes were crowded with basophils, so much so that chromophobes were difficult to find. In the center Gomori-positive basophils were present in greater numbers; a higher proportion than before were well filled with granules.

The presence of basophils of two distinct types in the central portion of the anterior lobe could be clearly demonstrated in Gomori-stained sections counter-stained by the modified trichrome method (Halmi, '50). The Gomori-positive basophils had angular shapes and vesicular nuclei. They were obviously outnumbered in the central area at this age by the small, round, Gomori-negative (and thus green-staining) basophils with shrunken cytoplasm and dense or pyknotic nuclei.

Day 28. The acidophils showed further development as compared with 21-day-old rats: their increased size often gave them oval or even cylindrical shapes. They still occurred in far smaller proportions than in the adult. Their nuclei were always round and vesicular.

The basophils in the "sex zone" stained more intensely at this time than at 21 days. This was due to the presence of more granules with strong PAS reaction. They were quite large, oval or round cells, arranged in strings or clusters along the blood vessels and containing vesicular or pyknotic nuclei. The basophils in the central area likewise were more prominent and clearly differentiated into two types. Those with angular shapes, resembling the thyrotrophs of the adult, were noticeably more common than they had been the previous week. They gave a positive aldehyde-fuchsin reaction, showing dark purple granules sometimes filling most of the cytoplasm and leaving the nucleus as a clear disc. When stained with PAS, the angular basophils gave a stronger reaction than did the rest. The latter were round or oval, and stained green with trichrome stain. Some of them had a shrunken appearance with a pyknotic nucleus and sparse cytoplasm. But others, not so small, showed a green, slightly granulated cytoplasm with vesicular but darker stained nuclei. These round, Gomori-negative basophils still outnumbered the angular Gomori-positive cells of the central area (figs. 9–12).

Weeks five to seven

Between day 35 and day 49 a remarkable cytological change took place in the anterior pituitary of the female rat. This change concerned all the granulated PAS-positive but Gomori-negative rounded cells in the "sex zone," along the pars intermedia and in the center of the pars anterior. At 35 days, 5 of the 12 female pituitaries examined showed almost complete degranulation of all these cells, which now appeared diffusely pink with PAS. In other animals (7 out of the 12), the peripheral cells had lost their granules, while the weaker-

staining granules in the central cells were still present. At day 42, the majority of females had gone through the process of degranulation, but large numbers of cells around the portal vessels were still diffusely pink. At 49 days, all gonadotrophs appeared grey and devoid of PAS-positive material. With Crossmon's stain the "sex zone" and marginal gonadotrophs at day 35 and even at 42 might still have a purple appearance comparable with that of day 28. The central gonadotrophs might be obvious by their light blue stain at days 35 to 42. However, on day 49 no "basophilia" (figs. 13, 15) could be demonstrated in the sex-region cells which rather resembled large "chromophobes."

The gonadotrophs in the male pituitaries, in complete contrast, showed increased numbers and size during this period. There was no evidence of diminished glycoprotein content, as in the female. On the contrary, the peripheral cells, including those in the "sex zone," contained intensely red-staining, coarse granules which are typical for the mature male (figs. 14, 16, 17). Such cells with dark granules could not be seen along the pars intermedia or in the center where the finely granulated PAS-positive, Gomori-negative "basophils" prevailed. The nuclei of the latter were mostly clear and vesicular at this stage (42 days). While they might be denser than the acidophil nuclei, they were now less frequently pyknotic.

The central region of both sexes, in addition to the cells just described, contained numerous well-granulated, Gomori-positive cells. They were now frequently arranged in groups, not connected to the larger vessels; they showed the typical bizarre shapes of the thyrotrophs and appeared to be present in greater numbers in the female than in the male.

The acidophils had not yet reached mature numbers, but their staining ability and sizes had increased considerably. Also, signs of function could be observed in the acidophils of the females. This will be pointed out in the description of the 56-day acidophils.

Week eight and onward

Day 56. With the exception of the pituitary of one female rat, the pituitaries of this group resembled closely those of normal, young, mature animals, of the respective sexes.

The "sex zone" of the male pituitaries contained large gonadotrophs which now were equal in number and of the same appearance as those found in the mature state. They were heavily granulated, oval in shape, with negative Golgi images and clear nuclei, and showed an affinity for blood vessels. Along the pars intermedia and spreading into the central region of the pars anterior, the round, lighter colored, Gomori-negative basophils showed increased size, negative Golgi images and clear nuclei (figs. 18–20). There were also abundant thyrotrophs at this age, most of which were filled with dark-staining granules.

The female pituitaries likewise showed abundant thyrotrophs (fig. 23). Their "sex zones" were typical of the mature female rat; that is, very few PAS-positive cells or "cyanophils" were found in the "sex zone," and they were small and pale. In the center, some round, shrunken basophils with pyknotic nuclei were seen.

Acidophils were now well developed in both sexes but there were noticeably fewer fuchsin-stained cells to be seen in the female. Such cells were frequently situated along sinusoids or "interlobular" septa, and in this way they appeared to encircle groups of "chromophobes," which were small, round or narrow cylindrical cells. In some female pituitaries, especially when observed in 2.5-μ sections, these nonstaining cells had enlarged negative images of the Golgi, which deviated from the circular or oval Golgi image of the basophils by being almost angular or shield-like in appearance and more closely attached to the nucleus. Such enlarged Golgi images were also seen in acidophils which still carried all or part of their granules and were lying in proximity to the described "chromophobes."

The preceding group, of 35–49 days, contained female pituitaries with changes in the acidophils similar to but not yet

so advanced as those described here for the 8-week-old rat. In rats younger than 35 days the changes were not seen.

Days 63, 70, and 77. Granulated gonadotrophs were consistently absent among the females of this group except for a few weakly-granulated basophils in one 9-week female. The male pituitaries were rich in gonadotrophs of typical morphology, location, and abundance (fig. 22).

Gomori-positive thyrotrophs in normal range of abundance were a constant feature of the pituitary sections in this group. The round, lightly-granulated basophil, a type which was present along the cleft and in the central area of the 56-day pituitaries, was encountered in sometimes astonishing numbers.

Acidophils of both males and females appeared to be equal in size and staining intensity.

DISCUSSION

This study shows that from birth through puberty the anterior pituitary cells undergo changes in morphology and relative proportions. Both acidophils and basophils — which in the first week of life were scarce, small, and indistinct — had, by the 14th day, increased both in stainability and in numbers. After the 14th day, acidophils changed gradually, attaining mature size and numbers at about two months. On the other hand, the number of basophils fluctuated during this period before finally becoming constant at two months. At 14 days, basophils approximated acidophils in number, and exceeded the proportions in which they occur in adult pituitaries. Between 14 days and 8 weeks, basophils increased in relative numbers and then decreased to normal adult proportions. During that period, the basophils continued to grow in size, and gradually assumed the shapes typical of mature cells of their particular kinds, until finally the appearance of the adult pituitary was achieved at 8 weeks.

The presence of two types of basophils in the anterior pituitary of the rat, as previously observed by Purves and Griesbach ('51a), has been confirmed. However, Purves and

Griesbach, examining young, mature rats, did not describe the PAS-positive, Gomori-negative basophil (cyanophil) cell, which abounds in the central area and along the cleft opposite the pars intermedia. This cell type is round, and often has a pyknotic nucleus which, in fixed and stained sections, is frequently separated from the scarce cytoplasm. In early stages of the rat's development, these cells are quite similar to those present in the "sex zone" and along the bigger vessels in the ventral sections. At 35 days, the latter cells frequently contain coarse, strongly PAS-positive granulated material, especially around the edge. Such cells are never seen in the central area, and it might be possible, with more experience, to link this cytological difference with a difference in function. In a recent preliminary publication, Purves and Griesbach ('52) have tried to throw some light on this subject:

"These central gonadotrophs which are not stained with aldehyde-fuchsin are prominent before the onset of sexual maturity in the female and degranulated just prior to the first ovulation. Degranulation of these cells is promoted by oestrogen injection which promoted storage in the peripheral gonadotrophs. It is possible that the central gonadotrophs are responsible for the luteinising hormone production while the peripheral gonadotrophs are responsible for follicle-stimulating hormone."

However this might be, on reaching the age of 35 days, the female rat starts to show degranulation of all the "gonadotrophs," and at 7 weeks, all females are practically free of granulated or even diffusely PAS-stained cells of the Gomori-negative category. In some females of 35 days it was noted that the peripheral gonadotrophs had degranulated prior to the central ones, while in others both regions showed loss of PAS staining at the time of examination. If it be assumed that degranulation in the FSH-secreting cells precedes that in the LH-secreting cells, then the interval between the occurrence of degranulation in these two types of cells might be 48–72 hours or even less. Therefore, weekly examinations as carried out in this study would not, in each case, reveal

the cytological "evidence" for the release of FSH and LH, i.e., degranulation of the peripheral gonadotrophs shortly before the central gonadotrophs, preceding the first oestrus. In the absence of information on this point, we are content to state that, coinciding with the first ovulation, there occurs a general loss of PAS-staining material from such cells as were considered to be related to sex function (Nukariya, '26; Catchpole, '49; Halmi, '50; Purves and Griesbach, '51). It seems certain that these cells in fact secrete the hormones which induce the first ovulation, and continue to regulate all future cycles of the female rat's life. In a recent paper, Mandl and Zuckerman ('52) found the onset of puberty in their albino rats to occur between 32 and 48 days, with a mean of 40.2 days. This is in good agreement with the early reports of Evans and Bishop ('22).

There is no doubt about the fate of the "sex zone" gonadotrophs during maturation and thereafter. In sections of the pituitary stained with periodate leukofuchsin, they can be seen in males of all ages, but they are absent in the females aged more than 35–42 days. The diagnosis of sex from a pituitary section alone is therefore quite feasible (after 49 days of age). Our knowledge about the central gonadotrophs is not so satisfactory. Within the frame of this investigation it may be said that the status of these cells parallels that of the peripheral gonadotrophs, i.e., at the age of 11 weeks they are still visible in the male but not in the female. Investigations on older animals are necessary for further information on this subject.

The cells staining with Gomori's aldehyde-fuchsin, which Purves and Griesbach ('51b) and Halmi ('52a) correlated to thyroid secretion, were named "thyrotrophs" by Purves and Griesbach. The cells are strongly PAS-positive and are easily recognized by their angular shapes, their occurrence in groups, and their strict absence from the "sex zone." This investigation has shown them outlined with granules as early as the first day after birth. They are not completely filled with granules, however, until a later age (about 28 days in both

sexes). From there on they become more numerous, darker staining, and resemble the picture in the adult animal. Their presence in the mature female at 49–56 days is more obvious than in the male of the same age, but it is unknown, as yet, whether this difference is a real one.

The scarcity of chromophils in the infant rat pituitary (first week of life) appears related to the late development of endocrine activity in this species. Rumph and Smith ('26) pointed out that thyroid development is much later in rats than in pigs, and later in pigs than in humans. Hall and Kaan ('42) demonstrated metabolic effects on amphibian larvae from the thyroids of fetal rats of 18 days gestation, whereas those of 17 days were inactive. Similarly, Gorbman and Evans ('43) showed that ability to accumulate radioiodine began in fetal rats of 19 days, becoming considerably stronger at 21 days. Thus, if thyrotrophin secretion is necessary for thyroid activity, the pituitary elaboration apparently takes place before birth, but not in amounts sufficient for storage of histologically demonstrable amounts of the hormone in the pituitary of the rat.

In a recent publication, Pearse ('52) reported the presence of PAS-positive cells in the pituitary of the human fetus at 19 weeks of age. This author did not discriminate between different functional types of "mucoid cells" or basophils.

We have seen that the acidophils in the rat take up their specific staining properties rather slowly. As judged by the number of fuchsin-stained cells, the acidophils attain the adult proportions far later than do the basophils. Their numbers are equal to those of the basophils for a considerable period of early life. It is possible, however, that some of the acidophils undergo a functional degranulation, e.g., when secreting growth hormone. This is definitely true for the maturing female pituitary, where partial or total degranulation was observed in some well-developed acidophils. Such cells showed the enlarged negative image of the Golgi appa-

ratus as described by Zeckwer ('44) and Bielschowsky and Hall ('51) for states of abundant oestrogen supply. It is our opinion that this phenomenon of acidophil degranulation plus Golgi enlargement, which is strictly confined to the female, is the result of follicle function and oestrogen release.

SUMMARY

1. A cytological study was made of the anterior hypophyses of rats of both sexes progressing in age from birth to maturity.

2. In keeping with recent knowledge, three types of cells containing glycoprotein and formerly summarized as "basophils" were followed, and certain patterns of development of each of these types have been described. Two distinct types of gonadotrophs, those in the periphery and those in the central portion of the anterior lobe, could be distinguished. The thyrotrophs were strictly confined to the central and posterior regions of the anterior lobe. The gonadotrophs preceded the thyrotrophs in development as judged by the presence of glycoprotein granules. These granules were first seen at day 7, but the positive cells were distinguishable from the first day of life by a diffuse pink color.

3. Between days 35 and 42, degranulation of gonadotrophs in the female took place, and this almost certainly was due to the release of the gonadotrophic hormones, coinciding with the first oestrus. In 7 out of 12 female rats, the degranulation of the peripheral gonadotrophs preceded that of the central ones. The possible significance of this phenomenon is discussed. From day 42 onward, there was no storage of gonadotrophic glucoprotein in the female.

4. The thyrotrophs, identified by their faculty of staining specifically with aldehyde-fuchsin, apart from giving a strong glycoprotein reaction with PAS, were recognizable from birth on. They were filled with specific granules only slowly, not reaching the aspect of the adult before 28 days.

5. Acidophil cells in small numbers could be identified by their staining reactions almost from birth. Full cell size and staining properties were not reached before 6–8 weeks. In the mature female, degranulated acidophils with enlarged Golgi images were observed from maturation onward.

LITERATURE CITED

BIELSCHOWSKY, F., AND W. H. HALL 1951 Carcinogenesis in parabiotic rats. Brit. J. Cancer, *5:* 331–344.

CATCHPOLE, H. R. 1949 Distribution of glycoprotein hormones in the anterior pituitary gland of the rat. J. Endocrinol., *6:* 218–225.

CROSSMON, G. C. 1937 A modification of Mallory's connective tissue stain with a discussion of the principles involved. Anat. Rec., *69:* 33–38.

EVANS, H. M., AND K. S. BISHOP 1922 On the relations between fertility and nutrition. J. Metab. Res., *1:* 319–333.

GOMORI, G. 1950 Aldehyde-fuchsin: a new stain for elastic tissue. Am. J. Clin. Path., *20:* 665–666.

GORBMAN, A., AND H. M. EVANS 1943 Beginning of function in the thyroid of the fetal rat. Endocrinology, *32:* 113–115.

HALL, A. R., AND H. W. KAAN 1942 Anatomical and physiological studies on the thyroid gland of the albino rat. Anat. Rec., *84:* 221–239.

HALMI, N. S. 1950 Two types of basophils in the anterior pituitary of the rat and their respective cytophysiological significance. Endocrinology, *47:* 289–299.

———— 1951 Further observations on two types of basophil cells in the anterior pituitary. Anat. Rec., *109:* 300.

———— 1952 Differentiation of two types of basophils in the adenohypophysis of the rat and the mouse. Stain Technol., *27:* 61–64.

———— 1952a Two types of basophils in the rat pituitary: "thyrotrophs" and "gonadotrophs" vs. beta and delta cells. Endocrinology, *50:* 140–142.

MANDL, A. M., AND S. ZUCKERMAN 1952 Factors influencing the onset of puberty in albino rats. J. Endocrinol., *8:* 357–364.

NUKARIYA, S. 1926 Über die Bedeutung der Rückresorption des Spermas und über mikroskopische Veränderungen der Hypophyse an jungkastrierten weissen Ratten. Pflügers Arch. f. d. ges. Physiol., *214:* 697–720.

PEARSE, A. G. E. 1952 The cytochemistry and cytology of the normal anterior hypophysis investigated by the trichrome periodic acid-Schiff method. J. Path. and Bact., *64:* 811–826.

PURVES, H. D., AND W. E. GRIESBACH 1951a The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus Hotchkiss staining for glycoprotein. Endocrinology, *49:* 244–264.

———— 1951b The significance of the Gomori staining of the rat pituitary. Endocrinology, *49:* 652–662.

PURVES, H. D., AND W. E. GRIESBACH 1952 Functional differentiation in the acidophil cells and the gonadotrophic basophil cells of the rat pituitary. Proc. Univ. of Otago Med. School, *30:* 27.

ROMEIS, B. 1940 Handb. d. mikrosk. Anat. d. Menschen. *6:* Part 3. S. Springer, Berlin.

RUMPH, P., AND P. E. SMITH 1926 The first occurrence of secretory products and of a specific structural differentiation in the thyroid and anterior pituitary during the development of the pig foetus. Anat. Rec., *33:* 289–298.

ZECKWER, I. T. 1944 Correlation of the alterations in mammary glands, pituitary body and ovaries of parabiotic rats. Arch. Path., *38:* 99–109.

PLATE 1

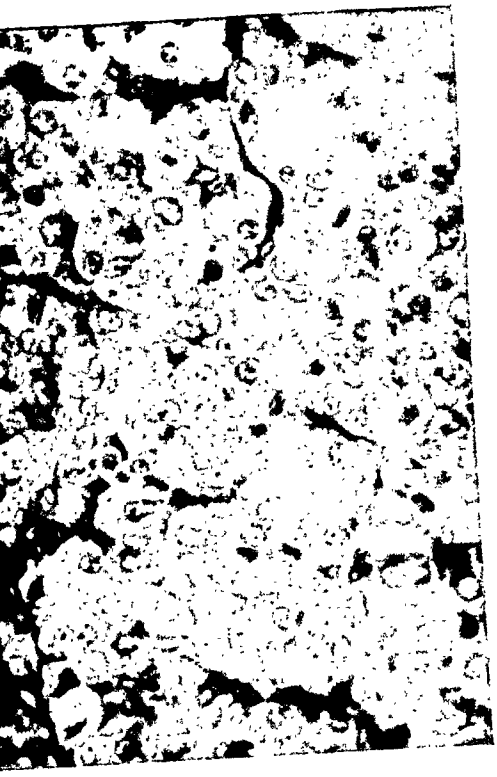
EXPLANATION OF FIGURES

1 Pituitary 1-day-old rat. Small cells consisting mostly of nuclei. Numerous mitoses. A few larger cells lie along sinusoids. PAS + hematoxylin, × 500.

2 Pituitary 3-day-old female rat. Anterior pituitary "sex zone" (right). Cleft free of colloid (center). Pars intermedia = P.I. (left). No cell differentiation visible as yet. PAS +hematoxylin, × 500.

3 Pituitary 3-day-old female. Diffusely PAS-positive cells along septa. Mitoses. PAS + hematoxylin, × 1100.

4 Pituitary 1-day-old rat. Aldehyde-fuchsin-positive cells in center, with rings of granules on cell periphery. Gomori, × 1100.



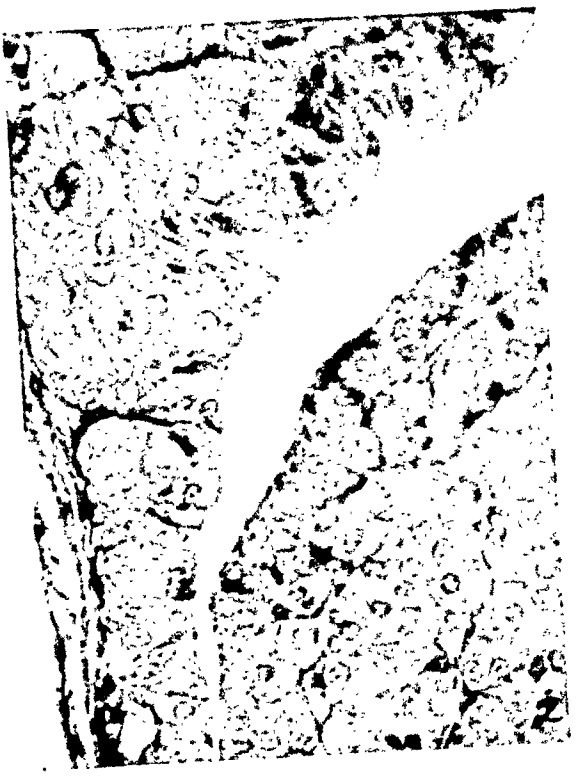



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PLATE 2

EXPLANATION OF FIGURES

5 Pituitary 5-day-old female showing small, dark, not granulated PAS-positive cells in "sex zone" bordering portal vessels. PAS + hematoxylin, × 840.

6 Pituitary 14-day-old female. Central area. Round gonadotrophs (grey) along sinusoids, with vesicular nuclei. A few angular thyrotrophs (black) in center of figure. PAS +hematoxylin, × 840.

7 Same section as in figure 6. "Sex zone" with scarcely granulated PAS-positive cells along vessels. PAS + hematoxylin, × 840.

8 Pituitary of 14-day-old male. Small PAS-positive cells bordering portal vessels. PAS + hematoxylin, × 500.

PITUITARY CYTOLOGY DURING GROWTH

E. SIPERSTEIN AND OTHERS

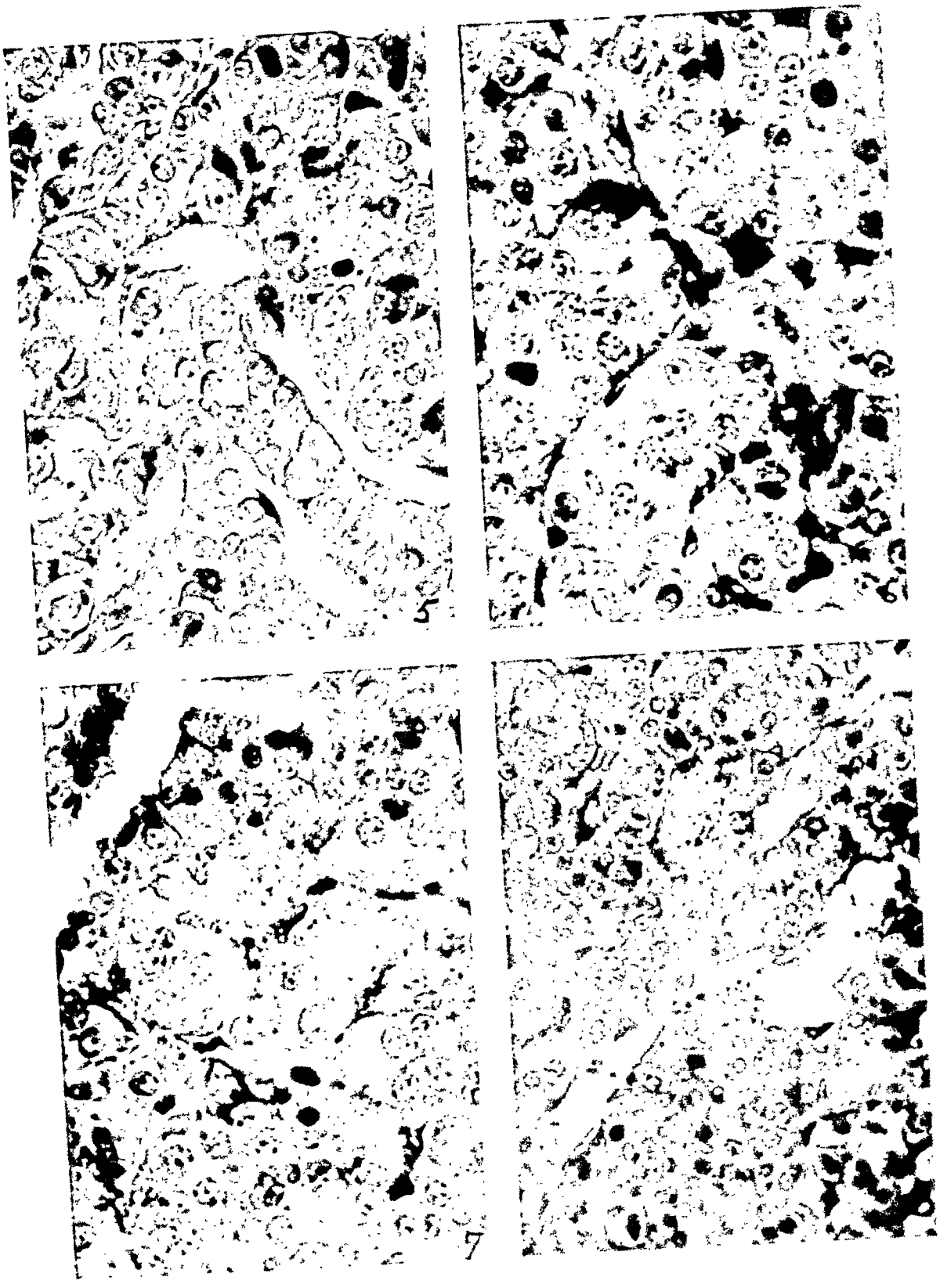


PLATE 3

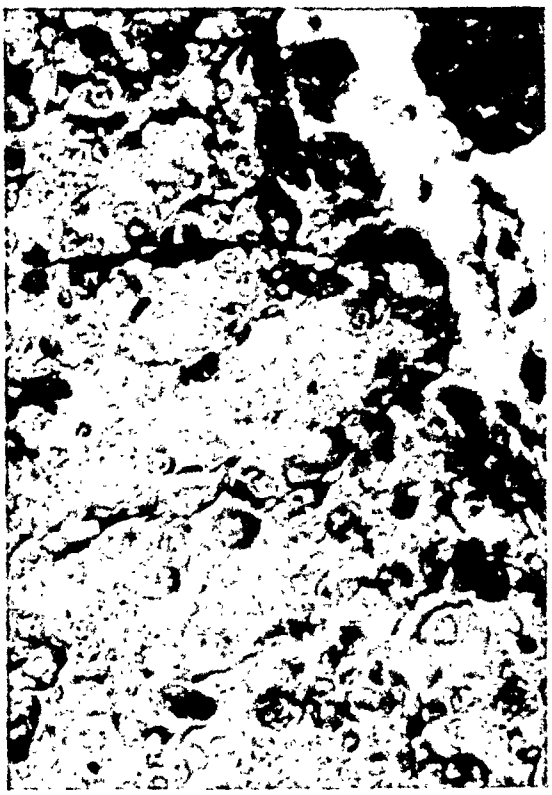
EXPLANATION OF FIGURES

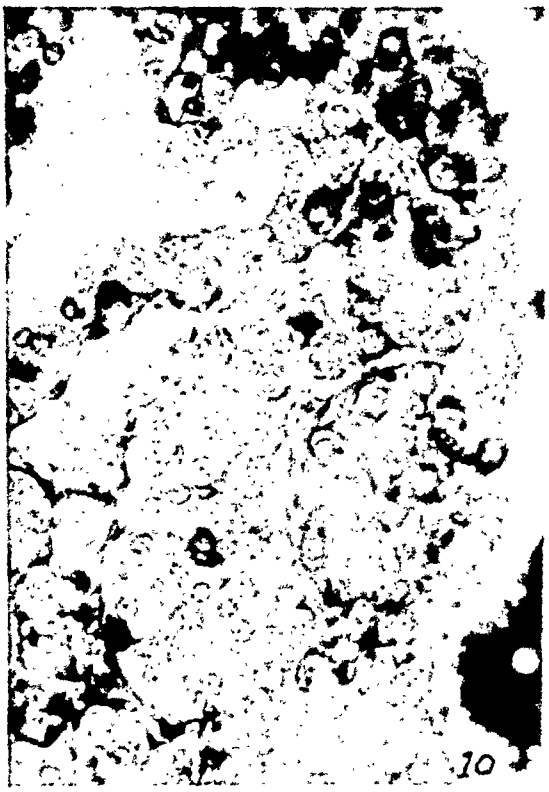
9 Pituitary of 28-day-old female. Sex zone, with small, round, darkly granulated gonadotrophs, plentiful along blood vessels. PAS + hematoxylin, × 500.

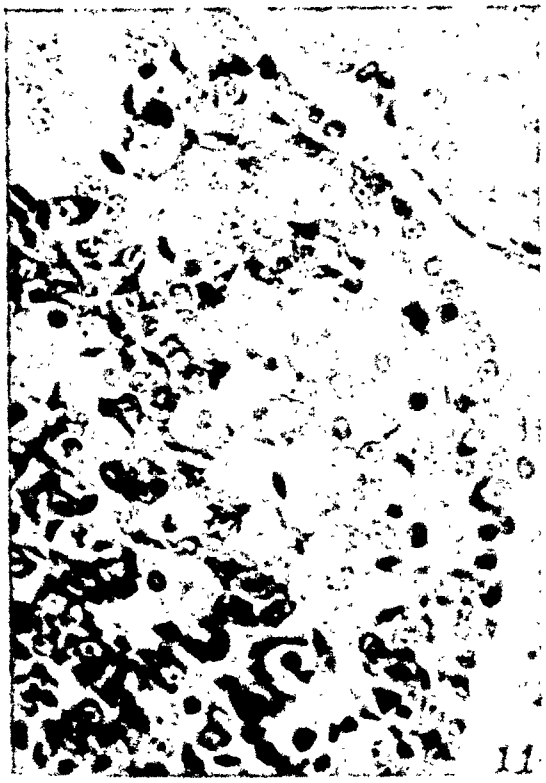
10 Pituitary of 28-day-old male. Sex zone. Development and number of gonadotrophs similar to female (fig. 9). PAS + hematoxylin, × 500.

11 Pituitary of 28-day-old male. "Sex zone" basophils appear light grey, with fine granules. Acidophils are small black cells. Crossmon. × 500.

12 Same pituitary and same area as figure 11. Large cells are gonadotrophs with fine PAS-positive granules. Vesicular and pyknotic nuclei are present. PAS + hematoxylin, × 500.







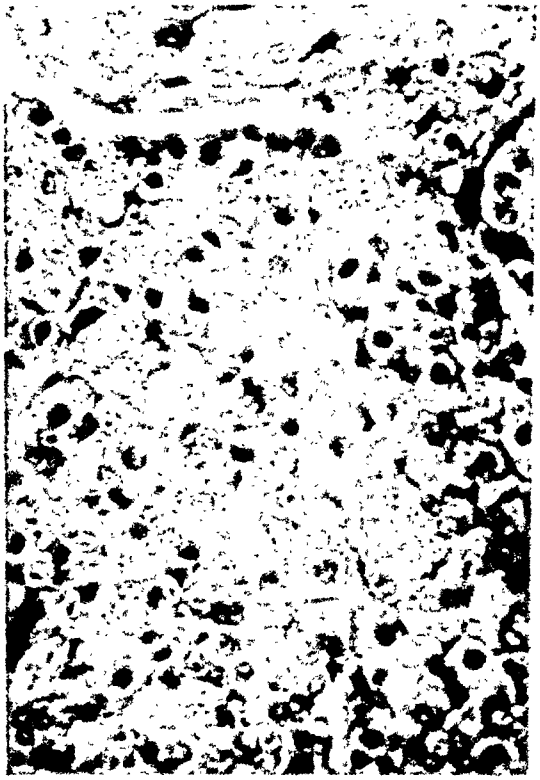


PLATE 4

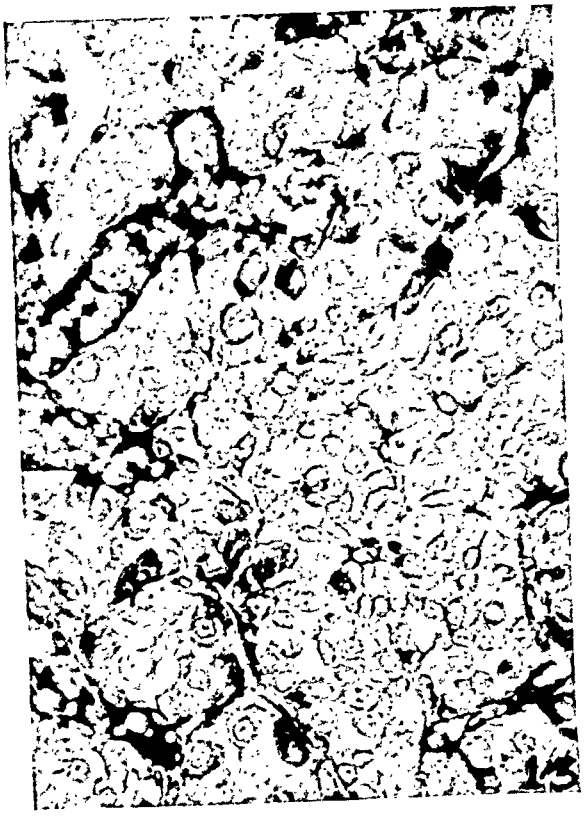
EXPLANATION OF FIGURES

13 Pituitary of 35-day-old female. Note large, degranulated gonadotrophs (grey) along portal vessels, and small, granulated gonadotrophs (black) bordering sinusoids. Illustrates early stage of degranulation in 5-week-old female. PAS + hematoxylin, × 500.

14 Pituitary of 35-day-old male. Granulated gonadotrophs of increased size along sinusoids. Compare with figure 13. PAS + hematoxylin, × 500.

15 Pituitary of 42-day-old female. Sex zone. Degranulation completed. Large, pale gonadotrophs may be identified along vessels. PAS + hematoxylin, × 500.

16 Pituitary of 42-day-old male. Striking contrast to figure 15. Gonadotrophs have further grown, some showing negative image of Golgi. Nuclei mostly vesicular. Fine granules which are darker within Golgi zone. PAS + hematoxylin, × 500.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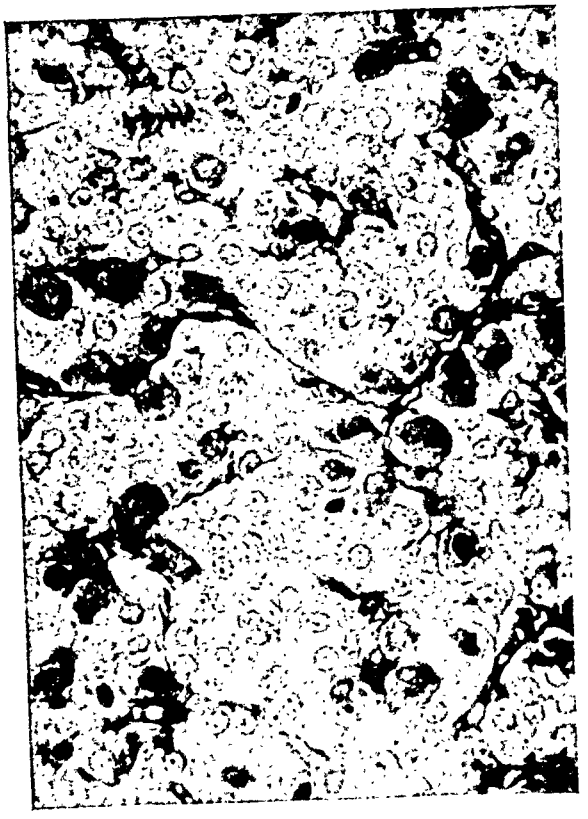

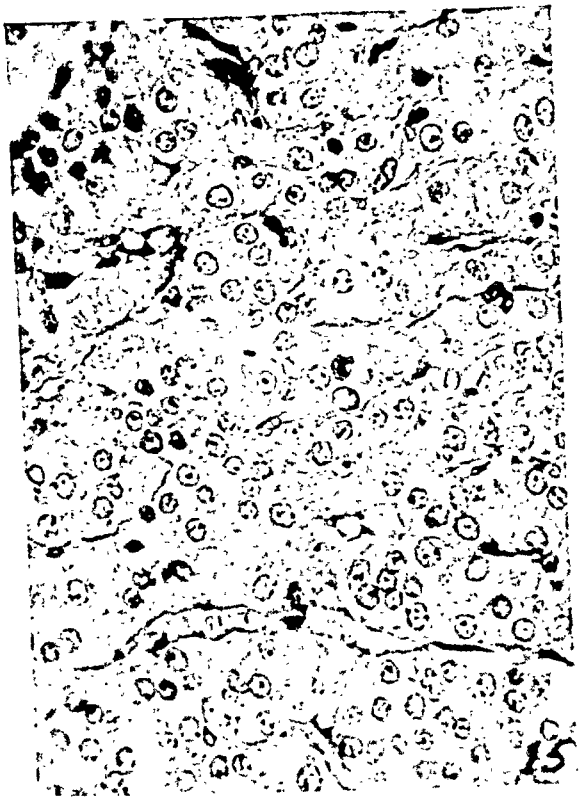
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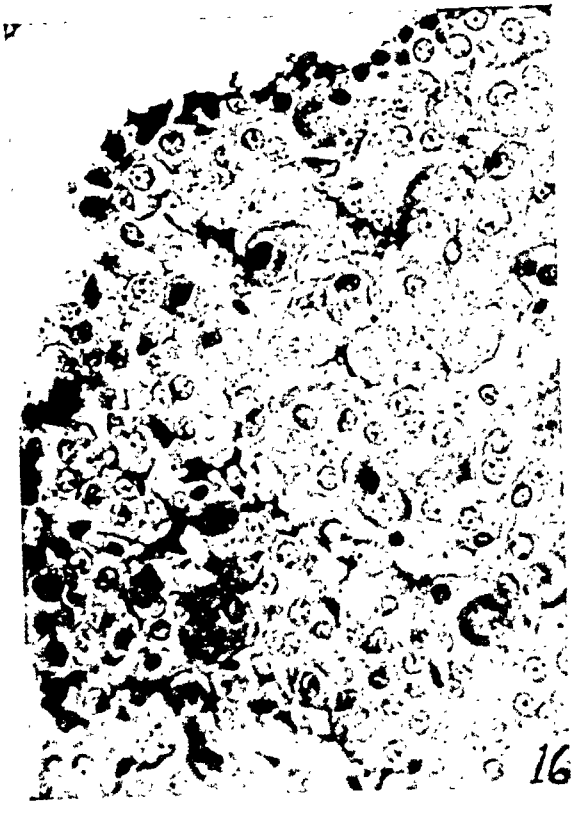

16

PLATE 5

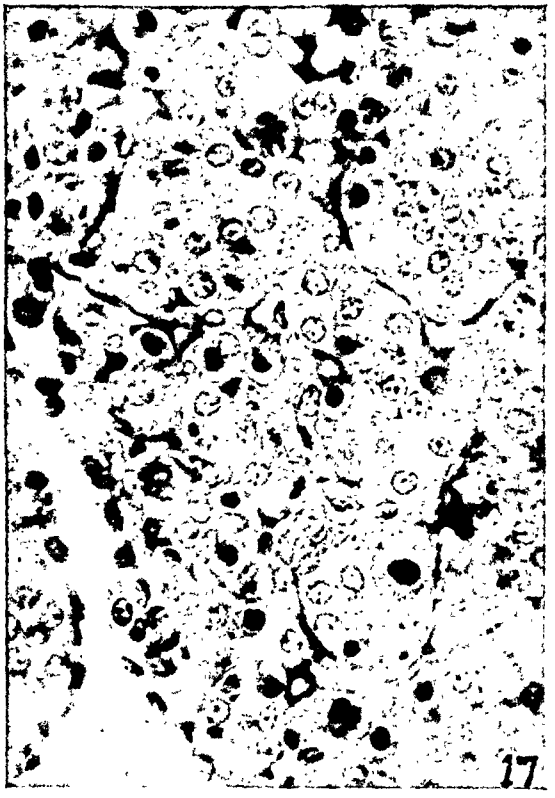
EXPLANATION OF FIGURES

17 Same section as in figure 16, closer to central area. Note pale central gonadotrophs along septa, and the small, round cells with pyknotic nuclei and shrunken cytoplasm. PAS + hematoxylin, × 500.

18 Pituitary of 56-day-old male (Wistar). High power view of sex zone gonadotrophs, illustrating the coarse, PAS-positive granulation along cell periphery. PAS + hematoxylin, × 840.

19 Pituitary of 56-day-old male (Sprague-Dawley). Sex zone with coarsely granulated gonadotrophs along blood vessels. Upper left shows colloid of cleft. PAS + hematoxylin, × 500.

20 Same section as figure 19, but closer to center, along the cleft and pars intermedia. Showing the pink, finely granulated, round, central type of gonadotroph, with vesicular nuclei and occasionally negative Golgi. PAS + hematoxylin, × 500.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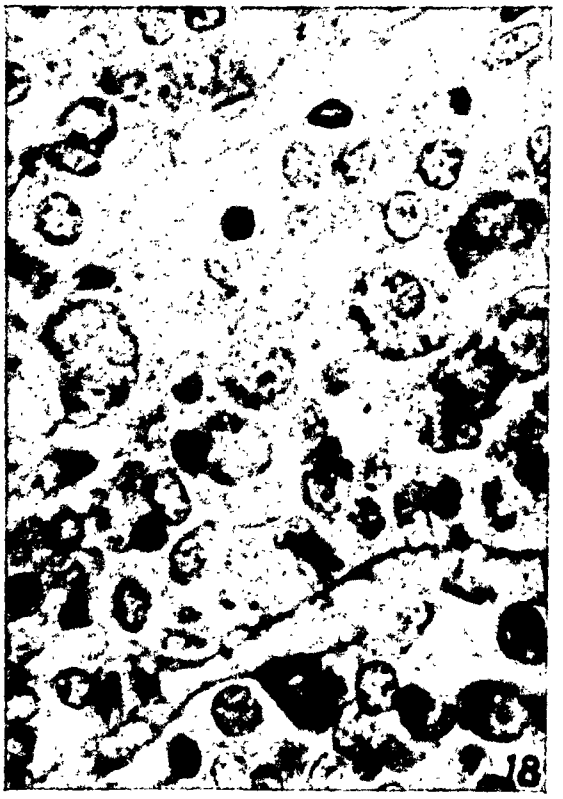
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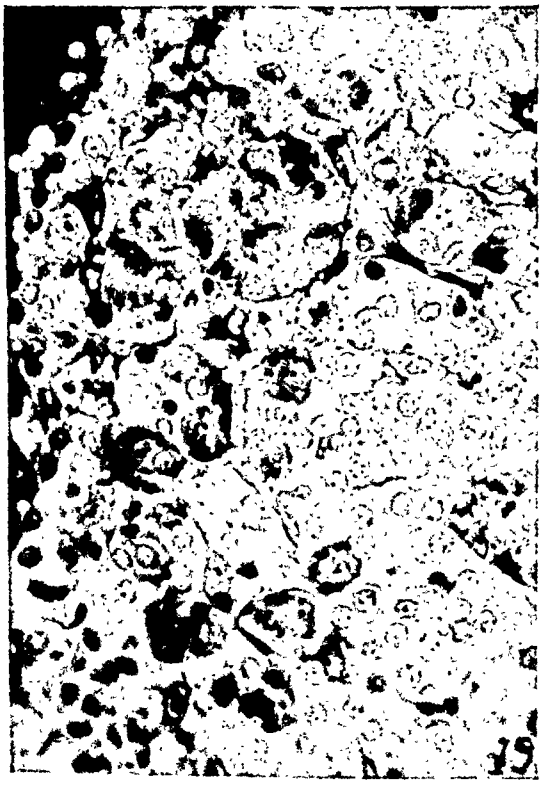
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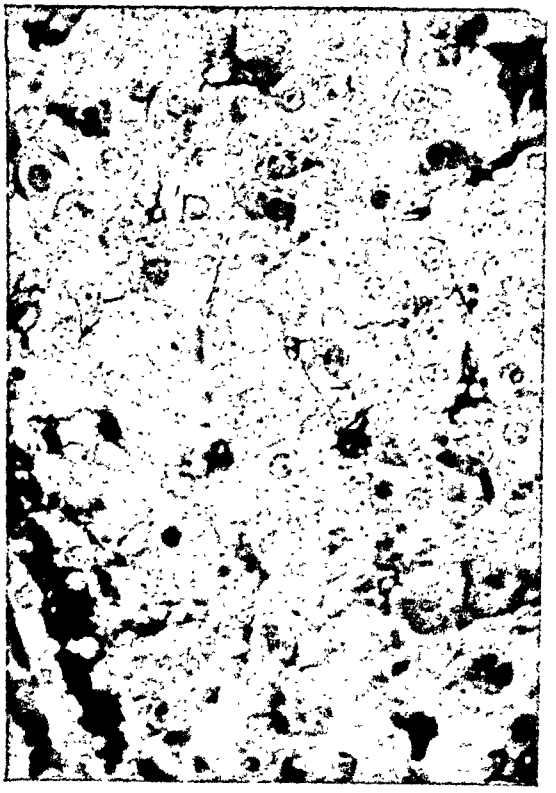


PLATE 6

EXPLANATION OF FIGURES

21 Pituitary of 70-day-old male. Central area with mature gonadotrophs along sinusoids. Golgi image marked. Some dark thyrotrophs of irregular shape. PAS + hematoxylin, × 840.

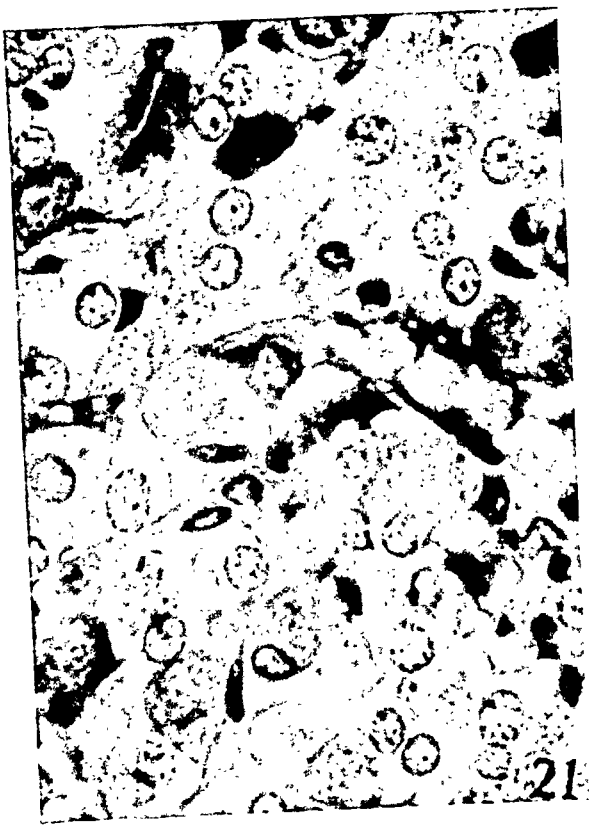
22 Pituitary of 63-day-old male. Mature gonadotrophs in sex zone, lying along vessels. The enlarged Golgi and the loss of coarse granules indicate a state of high activity. PAS + hematoxylin, × 1100.

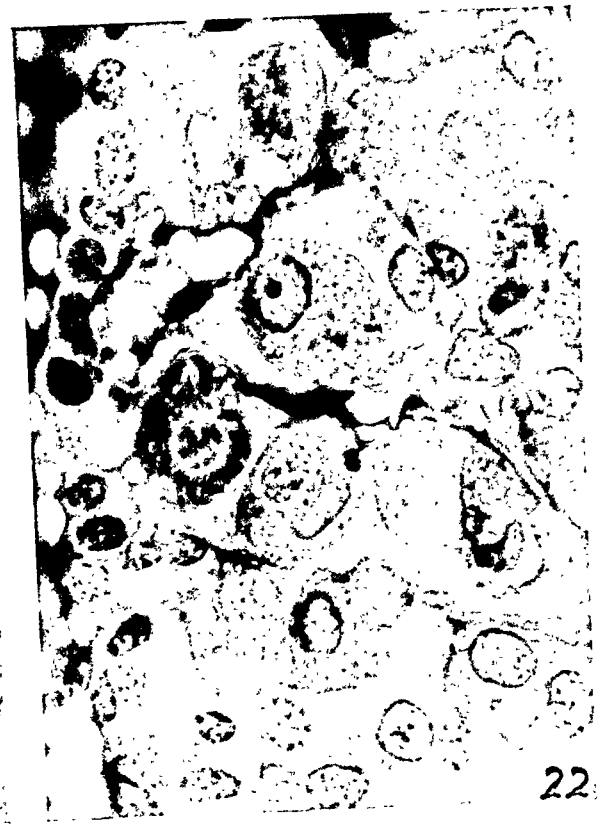
23 Pituitary of 63-day-old female. Fully granulated thyrotrophs from central area. Golgi just visible. In lower left along vessel some degranulated gonadotrophs. PAS + hematoxylin, × 1100.

24 Pituitary of 21-day-old male. Central area with groups of thyrotrophs, which at this stage are not completely filled with granules. The grouping of this cell type is typical for the thyrotroph of the adult age. Gomori, × 490.

PITUITARY CYTOLOGY DURING GROWTH

E. SIPERSTEIN AND OTHERS





AMERICAN SOCIETY OF ZOOLOGISTS

Officers for 1954

President . . . . . J. WALTER WILSON
Vice-President . . . . . FRANK A. BROWN, JR.
Secretary . . . . . S. MERYL ROSE
Treasurer . . . . . THEODORE L. JAHN

Executive Committee
(In addition to the four officers)

ROBERT CHAMBERS . . . . . to serve until December, 1954
ALFRED S. ROMER . . . . . to serve until December, 1955
D. M. WHITAKER . . . . . to serve until December, 1956
FRANZ SCHRADER . . . . . to serve until December, 1957
E. NEWTON HARVEY . . . . . to serve until December, 1958

Representative of the Society in the Division of Biology and Agriculture of the National Research Council

JOHN A. MOORE . . . . . to serve until July 1, 1956

Representatives of the Society on the Council of the American Association for the Advancement of Science

ROBERT C. MILLER . . . . . to serve until December, 1954
HAROLD H. PLOUGH . . . . . to serve until December, 1956

Representative of the Society on the Board of Governors of the American Institute of Biological Sciences

WALTER N. HESS . . . . . to serve until December, 1955

Representatives of the Society on the Advisory Editorial Board of Biological Abstracts

KARL P. SCHMIDT . . . . . to serve until December, 1954
G. FANKHAUSER . . . . . to serve until December, 1955
A. B. DAWSON . . . . . to serve until December, 1956
EDGAR ZWILLING . . . . . to serve until December, 1957

Representative of the Society on the Executive Committee of the Biological Stain Commission

To serve until December, 1954
F. H. J. FIGGE

Representative of the Society on the Board of Trustees of The American Type Culture Collection

To serve until December, 1954
R. P. HALL

Managing Editor of the Journal of Morphology

E. G. BUTLER . . . . . to serve until December, 1954

Members of the Editorial Board of the Journal of Morphology

To serve until December, 1954

C. LADD PROSSER — ELEANOR H. SLIFER — TALBOT H. WATERMAN

To serve until December, 1955

DWIGHT E. MINNICH — BERWIND P. KAUFMANN — J. WALTER WILSON

To serve until December, 1956

E. R. BECKER — PERRY W. GILBERT — LIBBIE H. HYMAN

Notice

For a list of the names of the former officers of the Society, including representatives of the Division of Biology and Agriculture of the National Research Council, the associate editors of the Journal of Morphology and the constitution of the Society, see the Anatomical Record, Vol. 112, No. 3, 1952.

IN MEMORIAM

EDWIN GRANT CONKLIN

1863 – 1952

PROCEEDINGS OF THE FIFTIETH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOOLOGISTS

HOTEL STATLER, BOSTON, MASSACHUSETTS
AND
THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

CAMBRIDGE, MASSACHUSETTS

DECEMBER 28, 29, 30, 1953

PROGRAM

The Annual Meeting of the American Society of Zoologists was held in Boston and Cambridge in conjunction with Section F of the American Association for the Advancement of Science. The meetings for the reading of papers and the Presidential Symposium were held in the Hotel Statler. The demonstrations, motion pictures and the panel discussion on "The Teaching of Physiology at the Undergraduate Level" were held in the new Dorrance Building of the Massachusetts Institute of Technology.

The Boston-Cambridge meeting was unusually well attended. Discussion at the paper reading sessions was often lively and stimulating. The Presidential Symposium on "Bioluminescence as a Tool in the Study of Cell Processes" attracted a large audience. Dr. Paul Weiss, Vice-President of the American Association for the Advancement of Science and Chairman of Section F, delivered an entertaining and thoughtful dinner address.

Last year the number of papers at the September meeting in Ithaca reached a low for recent years. The number was 188. This year there was a return to a more normal number, 268. The figures for the years 1950 and 1951 were 250 and 298.

BUSINESS MEETING

The fiftieth annual business meeting was called to order at 4:50 P.M. on Monday, December 28 by President E. Newton Harvey.

The following business was transacted

1. The minutes of the last meeting. These were published in the December 1952 supplement of the Anatomical Record and distributed to all members of the Society. It was voted to approve the minutes as printed.

2. Memorial resolutions. The following resolutions were prepared:

JAMES WILLIAM BUCHANAN

1888 – 1952

In the sudden and unexpected death of Dr. James William Buchanan of a heart attack at his home in Los Angeles on June 27, 1952, American zoology lost a widely known and highly esteemed member.

Dr. Buchanan was born on January 30, 1888, at Basil, Ohio, and did his undergraduate work at Ohio University (Athens) where he took the B.S. degree in 1913, specializing in zoology. He taught at the University of Mississippi for two years and at New York University for a year before serving in World War I as a first lieutenant of infantry overseas and then with the army of occupation in Germany. He was senior instructor and organizer of the 3rd Army post schools in Coblenz, Germany, in 1918 and 1919, and then studied at the University of London in 1919. He took his Ph.D. degree at the University of Chicago under Professor C. M. Child in 1921, thus indicating a taste for the physiological aspects of zoology. This training led to his appointment at Yale University in 1921, primarily to take over a course in General Physiology, secondarily to cooperate in the teaching of General Biology. In 1930 he went to Northwestern University as associate professor of zoology, rapidly advancing to full professorial rank (1933), and served as chairman of the department of zoology there from 1941 to 1949. While he was active in research at Northwestern, his fine administrative ability was soon recognized and he was constantly called upon to serve on the most important administrative committees of the university. Recognition of his executive and organizational abilities finally led to his appointment as acting dean of the college of Liberal Arts at Northwestern during his last years there. Buchanan was in large part responsible for many improvements in the zoology department at Northwestern.

His outstanding executive and administrative capacities led to his appointment as director of the Allan Hancock Foundation at the University of Southern California following his retirement from Northwestern in 1949. He was simultaneously

chairman of the College of Letters, Arts, and Sciences at Southern California. He won immediate acclaim in his new positions and was making rapid progress in supervising the research work and generally revising the projects of the Hancock Foundation at the time of his death.

Buchanan published a number of research articles in the twenty years following his taking of the doctor's degree. These were concerned chiefly with pursuing various lines of inquiry suggested to him by his association with Professor Child, mostly with investigations of the nature of disintegration and regeneration gradients.

Buchanan is survived by his wife and two sons, James, 33, an assistant professor of physics at West Virginia University, and William, 28, who recently returned home from army duty in Japan. William was also a lieutenant in the Army of Occupation in Germany following World War II, thirty years after his father served similarly there.

Perhaps Dr. Buchanan's outstanding characteristic was a dry and rapid-fire wit, to which perhaps an edge was given by the subnormal health from which he suffered during much of his life. His army service and teaching experiences in a variety of places enabled him to make pithy and caustic comments on almost any topic. He saw through every sort of sham to the heart of a situation and no doubt this quality was at the bottom of his highly successful handling of university and scientific affairs. His amusing conversation and outspoken letters will be long remembered by a large circle of friends.

LIBBIE H. HYMAN

EDWIN GRANT CONKLIN

1863 – 1952

With the death of Edwin Grant Conklin on November 21, 1952, just before his eighty-ninth birthday, the American Society of Zoologists has lost one of its most distinguished members, and biology one of the great interpreters of the science. Although not a charter member of the Morphological Society, founded in 1890 and predecessor to the American Society of Zoologists (founded 1903), Professor Conklin became a member in 1896 and its President in 1899. He took an active interest in its affairs and served on many of its committees.

In the Morphological Society he was associated with that group of American biologists who represented every branch of the subject and so markedly influenced the course of zoology in the United States. E. B. Wilson, C. O. Whitman, E. L. Mark, C. S. Minot, H. F. Osborn, T. H. Morgan, J. S. Kingsley, H. C. Bumpus, W. B. Scott, S. I. Smith, W. M. Wheeler, E. A. Andrews and G. H. Parker were officers of the early society, while C. B. Davenport, F. H. Herrick, S. Watase, W. Patten, B. Dean, F. R. Lillie, J. P. McMurrich, J. Reighard, C. W. Hargitt, H. S. Jennings and R. G. Harrison were at some time during its existence members of the Executive Committee. All these men were his friends. They represent the generation which continued the work started by Louis Agassiz at Harvard in the middle of the last century. Professor Conklin was a leading member of those

scientists who explored the little known fields of embryology and cytology, stimulated by the rapid expansion of zoological knowledge.

Throughout his life Professor Conklin was associated with learned societies and learned publications. The Journal of Morphology was founded in 1887 and he became an associate editor in 1900. His name first appears as editor of the Biological Bulletin in 1902. At the beginning of the twentieth century, zoological investigation became more experimental and in 1904 the new Journal of Experimental Zoology was founded, with Professor Conklin on its editorial board. He was a "charter editor" of Genetics in 1916 and was chosen a member of the advisory board to represent embryology for the newly formed Quarterly Review of Biology in 1926.

Editorship was only one outlet for Professor Conklin's energy. Throughout his long and active life he contributed over two hundred papers to various journals and wrote ten books. Many honors came to him. He was elected a member of eight American scientific societies or academies, and President of four of them; was a foreign member of eight European societies or academies; a trustee of seven American institutions and President or Vice-president of three of them. He occupied professorships in four Universities and received eight honorary degrees. Such is a short roster of accomplishments of one of our most distinguished members.

It is fortunate that among Professor Conklin's valuable attributes was a prodigious memory for detail, perhaps fostered by his thesis study of cell lineage. Even during the later years of his life, the date of almost any event was recalled with precision and those of us who knew him well, were entertained by many an amusing anecdote of early life in the Middle West and of his later educational period.

He was born at Waldo, Ohio, on November 24, 1863, the son of Maria Hull and Abram V. Conklin, a country doctor. I recall the year he reached the age of 87 and referred to the words, "Four score and seven years ago," which Lincoln spoke at Gettysburg on November 19, 1863, five days before his birth, and to the fact that two long human lives could reach back to 1776 and the Declaration of Independence.

Like Lincoln, his early life was connected with the development of the midwest. As a child he had traveled from one part of Ohio to another in a covered wagon, as a boy had worked on a farm, and attended a country school of one room and one teacher. Later he became the teacher in just such a country school, with duties not only of instructor but of janitor and disciplinarian, at a salary of $35 a month.

After graduating from high school at Delaware, Ohio, he attended Ohio Wesleyan, obtaining a B.S. degree in 1885 and a B.A. in 1886. It is here that he first became interested in Science, fostered by collecting trips for shells and fossils under the influence of the Professor of Biology and Geology, Edward T. Nelson. Professor Nelson turned him toward biology and the experience of the next three years teaching Latin, Greek and Science at Rust University, a Missionary College in Mississippi, matured the decision to make biology his life work. He entered Johns Hopkins University Graduate School in 1888 and started work with W. K. Brooks. The first problem was the identification and morphology of a siphonophore, collected by Alexander Agassiz in the Pacific. For continuation of these studies, he was sent to the U. S. Fish Commission Laboratory at Woods Hole, Mass. Perhaps it is fortunate that there were no siphonophores to be obtained there. Turning to the varied fauna of that region, the abundant embryological material attracted him,

and thus began the extended studies on cell lineage, first of Crepidula, which was to be incorporated in his thesis, followed by a similar investigation of ascidian eggs, and later of Amphioxus. In connection with the developmental history of the regions of the egg, or of the blastomeres, the details of cell structure and of cell division were studied in the greatest detail and later many experiments carried out to discover what influence environmental changes might have on the cytology. Embryology and cytology were his chosen fields for investigation.

Space does not permit a detailed account of the early influences in Professor Conklin's life, so important in moulding his personality. Suffice it to say that the religious influence was strong in his home and in Ohio Wesleyan. Perhaps he just escaped being a preacher. The ability to quote scripture served him well in later years as a popular lecturer. The controversy over evolution was at its height in his early manhood. He studied Darwin's books carefully and became convinced of the truth of this great principle which he always regarded as the central theme of biology. It is not surprising to find that part of the agreement before he later accepted a teaching position was that he must present his own beliefs regarding evolution, without censure or dictation from the University.

Professor Conklin's various positions acquainted him with diverse regions of the United States. His early experience (1885–1888) at Rust University in Mississippi, a Negro school in the far South, served to instill qualities of tolerance and self-reliance, which were so characteristic of all his actions. After receiving the PhD. at Johns Hopkins in 1891, he became successively Profesor of Biology at Ohio Wesleyan (1891–1894), Professor of Zoology at Northwestern (1894–1896) and at Pennsylvania (1896–1908), and finally Chairman and Professor of Biology at Princeton University (1908–1933). His retirement at Princeton in 1933 involved little change in activity. He took a continued interest in departmental affairs, giving seminars for graduate students, in addition to writing ninety-three articles for journals, magazines or publications of learned societies.

A broad interest in Science resulted in early election to the American Philosophical Society (1897) and the National Academy of Sciences (1908). The Philosophical Society "held at Philadelphia for promoting useful knowledge" particularly appealed to him because of the wide range of subjects discussed. He rarely missed a meeting and took the most active part in its affairs, serving on committees, as Councillor, Executive Officer, and President for two terms, 1942–1945 and 1948–1952.

Although Professor Conklin's special field of investigation was the cell, his interest in biology was the whole organism, particularly man himself The egg of the ascidian and especially the embryology of Amphioxus may have appealed to him because of their status as Protochordates, ancestors of the vertebrates and thus of man.

Man's place in nature and the broader implications of biology occupied his thoughts more and more as his career progressed. This trend is especially noticeable in the titles of his books. The most important book, a best seller, was "Heredity and Environment," first appearing in 1914. It passed through six editions and was translated into French, Japanese and Russian. Titles of his later books are "Direction of Human Evolution" (1920, 1922), "Mechanism of Evolu-

tion'' (1920), ''Morphology of Animals'' (1927), ''Problems of Development'' (1929), ''Freedom and Responsibility'' (1935), ''Science and Ethics'' (1937), ''Biology and Democracy'' (1938), ''What is Man?'' (1941) and ''Man: Real and Ideal'' (1943).

Throughout his life, the human interest led to acceptance of executive duties, to willingness to serve on many committees, to the espousing of various causes, all of which took time away from scientific research, but served as the background for a far wider viewpoint than specific research would have given. He was especially interested in education and in the philosophy of religion. Commencement addresses and published pamphlets present an original viewpoint in these fields. Always a liberal in outlook, he was a great believer in freedom, and, like most scientists, was vehemently opposed to any sort of regulation and regimentation.

Apart from a great loyalty to Princeton University, Professor Conklin had three outside interests to which he devoted a great deal of thought and to which his service may be said to have been really dedicated. First and foremost was the American Philosophical Society mentioned previously. Another was the Marine Biological Laboratory at Woods Hole, with which he had been connected almost since its beginnings in 1888. He started teaching there in 1891, and served as trustee since 1897. At the ''M.B.L.'' the atmosphere was, and still is, conducive to scientific freedom in its broadest sense: no direction of what the inveestigator should study, no pressure to produce premature results, only the attempt to supply the living material and the best conditions for research.

The third was the Bermuda Biological Station for Research in which Professor Conklin had as personal an interest as its founder, Professor E. T. Mark, who was for many years its Director. On reorganization of the Bermuda laboratory in 1926, Professor Conklin was made a trustee and served as President of the Corporation from 1926 to 1936. The Academy of Natural Sciences of Philadelphia and The Wistar Institute of Anatomy were two more institutions which occupied a great deal of his time.

As committeeman, he was a persuasive speaker and as lecturer an eloquent one. The human interest made the general biology lectures at Princeton University a popular course for many years, and he was in great demand for talks in which science is interpreted for the layman. His long association with Science Service (President, 1937–1945) and the American Association for the Advancement of Science (President, 1936), again reflect the broad interest in science and man.

Professor Conklin liked nothing better than to gather around him, in the laboratory or at his home, a group of students, for discussion of various subjects. These were times for reminiscence, during which one could learn much of the history of American Biology in the early part of the 20th century, made colorful by the personalities of such men as Loeb, Morgan and Wilson. His love of social contacts was ably supported by his wife, the former Belle Adkinson, who was always interested in his many friends and was a delightful hostess. Members of the American Society of Zoologists and all who knew him intimately will mourn the loss of one of the truly great biologists.

E. Newton Harvey

OTTO CHARLES GLASER

1880 – 1951

Otto Charles Glaser, Harkness Professor of Biology Emeritus at Amherst College, died in Northampton on February 10, 1951. He was seventy years old on the 13th of October 1950, and had been Professor of Biology at Amherst College for thirty-three years, ever since transferring from the faculty of the University of Michigan in 1918. He came to Amherst as a vigorous young teacher of thirty-eight. He was handsome, likable, and known favorably to many of the leaders in the field as an independent and promising thinker. He possessed an infectious enthusiasm for teaching biology to all undergraduates as an important educational experience, an essential aid in understanding their own limitations and capabilities in the twentieth century world. He was brought to Amherst by President Meiklejohn for precisely these reasons, and right up to the time of his death, his educational ideals and enthusiasms remained essentially unchanged.

Otto Glaser was born in Wiesbaden, Germany, in 1880, and in infancy was brought to this country when his parents emigrated to Baltimore. His father was a successful industrial chemist, and it is not surprising that both Otto and his younger brother, Rudolph, became scientists. Otto was always a precocious young man. He went to the Baltimore public schools, and before he was twenty was graduated from the undergraduate college of Johns Hopkins University. The same year he was accepted as a graduate student in zoology at Hopkins under the famous teacher W. K. Brooks, and he was the last of Brooks's students, for this great teacher died in 1904. In a seminar about two years ago Glaser told a group of us with a good deal of nostalgia about his days in Brooks's laboratory. Even though equipment was lacking in those days Brooks's personality dominated all of his students, and they left him with an enthusiasm for independent study and investigation which carried over with Glaser as with many others through their active lives as scientists.

Brooks's students were always in demand, and after taking his Ph.D. in 1904, several months before his twenty-fourth birthday, Glaser went to the University of Michigan as instructor in zoology. There he was advanced to Associate Professor by 1918 when he came to Amherst.

During his early days at Michigan Glaser made his acquaintance with the Marine Biological Laboratory at Woods Hole. At Woods Hole his broad scientific interests and wise counsel were widely appreciated. He was made a trustee of the institution in 1926, and remained on the board until his death.

Otto Glaser was an active investigator in biology throughout his life, and he never lost contact with important new trends as they appeared. Nevertheless, his most important influence, and that which claimed his major enthusiasm, was as a teacher. He was anxious to have all students generally appreciate the major contributions of science, and their philosophical and humanistic implications. One of his early papers was entitled "Is a scientific explanation of life possible?", and he never really left that problem. At Michigan he developed a very successful course in heredity which was open to all students without scientific preparation. In it he gave the essential facts as they were known then, but he went on to attack and to try to solve the problem of individual determinism which the realization of the

limitations of an individual's own genetic endowment imply. The humanizing of biology was Otto Glaser's major interest, and it found its outlet in Amherst especially in introductory biology (Biology 1). His influence will continue through Amherst graduates of the last thirty college classes for many years to come.

H. H. PLOUGH

GILBERT LOGAN HOUSER

1866 – 1951

In the passing of Gilbert Logan Houser on July 16, 1951, the American Society of Zoologists lost one of its oldest members and the State University of Iowa the last of the founders of its department of Zoology, a distinguished teacher and an efficient student of biological problems.

Gilbert Logan Houser was born on a farm in Lee County, Iowa, on July 9, 1866. He attended Whittier College of Salem, Iowa and Howe's Academy and the Iowa Wesleyan College of Mt. Pleasant, Iowa before coming to the University of Iowa, where he received his B.S. in 1891 and his M.S. in 1892. His Master's thesis was done on the Dolomites of the Niagara of Iowa and was published in the Iowa Geological Survey, Vol. 1, 1893. He was made instructor in the Department of Natural History at the State University of Iowa in 1892, under Calvin. He became a fellow of the Iowa Academy of Science in 1893. In 1893 he published two papers in Volume 4 of the Proceedings of the Iowa Academy of Science: one on "The uses of formaldehyde in animal morphology," and the other, a forecast of his doctoral research, "The nerve cells of the shark's brain." He completed his Ph.D. at Johns Hopkins in 1901, and his thesis on the neurones of the selachian brain was published in the Journal of Comparative Neurology, Vol. X1 (1901).

He was made Assistant Professor and head of the Department of Animal Morphology and Physiology in 1895, which department, in 1905, became united with Systematic Zoology to form the Department of Zoology, under Nutting as head. In 1893, together with Wickham, he helped Nutting organize the famous Bahama Expedition, which brought a wealth of deep-sea material to the University of Iowa Museum of Natural History. He was collaborator on the American Journal of Anatomy and on the Journal of Applied Microscopy in their early days. He was a member of several national scientific societies, did work on the Iowa Geological Survey, and was president of the Iowa Academy of Science in 1910. His special interest in research was in the fields of experimental biology, cytology, and the nervous system of lower vertebrates, in which he directed research and published several papers. As a teacher and director of research he was meticulous and painstaking, and expected his students to measure up to his standards. His many students remember with pleasure and profit his clear cut, well organized lectures and laboratory techniques.

His wife, Hattie Briggs Houser, preceded him in his passing by a few hours; they are survived by five sons.

ROBERT L. KING

HOWARD MADISON PARSHLEY

1884 – 1953

Dr. Howard M. Parshley, Professor Emeritus of Zoology at Smith College, died of a heart attack on May 19, 1953. He is survived by his wife, a son and daughter, both married, and five grandchildren.

Dr. Parshley was born August 7, 1884 at Hallowell, Maine and educated at the Boston Latin School, Harvard University (A.B., 1909, A.M., 1910, Sc.D., 1917) and the New England Conservatory of Music (1906–1909). He was Instructor in Zoology at the University of Maine from 1911 to 1914 and came as an assistant professor to the Department of Zoology at Smith College in 1917. In 1919, he was advanced to an associate professorship and became a full professor in 1925 serving in that capacity until his retirement in 1952. At Cold Spring Harbor, Dr. Parshley held the position of Associate Field Zoologist during the summers of 1919–1925 and, in the summer of 1927, was Field Zoologist and Entomologist at the University of Chicago.

Working under Professor W. M. Wheeler at the Bussey Institute (Harvard), 1914–1917, Dr. Parshley further developed his boyhood interest in entomology. He became an international authority on the Hemiptera and managing editor of the Catalogue of the Hemiptera. In addition to his publications in this field, he built up a valuable collection of entomological specimens, particularly of the Hemiptera, which was often studied by workers on that group. This collection is now located at the California Academy of Sciences in San Francisco.

In his thirty-five years of teaching at Smith, Dr. Parshley developed courses in general zoology, entomology and genetics. Through genetics, he became interested in problems of human reproduction which led to the publication of *Science and Good Behavior* (1928) and the *Science of Human Reproduction* (1933). *The Second Sex* by Simone de Beauvoir, published in the U. S. A. in 1953, was edited and translated by Dr. Parshley and he had the satisfaction of knowing of its success. At the time of his death, he was awaiting page proof of his translation of D. F. Bourlier's *Vie et Moeurs des Mammiferes*. Other publications include the *Survey of Biology* (1940), fifty-six papers on the Hemiptera, and over three hundred miscellaneous articles and reviews.

He was a member of the American Society of Zoologists, the Entomological Society of America, the Genetics Society of America, the American Society of Naturalists, Societé Entomologue de Belgique, Sigma Xi, A. A. A. S., and Phi Beta Kappa.

In addition to his scientific work, Dr. Parshley had an equally great interest in music. For thirty-six years he played the double bass in the Smith College Symphony Orchestra; he was a charter member of the Springfield Symphony Orchestra and a member of the Pioneer Valley Symphony Orchestra. During his last week, he had taken part in two concerts and had attended a college symphony rehearsal on the evening before his death.

At Smith, where he served a number of terms as chairman of the Department of Zoology, his good judgment and fairmindedness were much appreciated by his colleagues. Students found him an authority on the exact meaning of scientific

terms, because of his excellent training in classics, and he was generous of his time when consulted on points of style in writing. Dr. Parshley was an interesting and a friendly man who gave wise counsel on many matters to those who sought it from him.

LOIS E. TE WINKEL

HARLEY JONES VAN CLEAVE

1886 – 1953

Professor Harley Jones Van Cleave died on January 5, 1953, after suffering from carcinoma for several years. He was born at Knoxville, Illinois, on October 5, 1886, and in 1909 received his B.S. degree from Knox College at Galesburg, Illinois. He entered the graduate school of the University of Illinois at Urbana and was awarded the degrees of M.S. in 1910 and Ph.D. in 1913. After receiving the doctorate he was invited to become a staff member of the Department of Zoology of the University of Illinois where he remained until his retirement in 1952. Dr. Van, as his students affectionately knew him, was an indefatigable worker who led an extremely active life until confined to his bed shortly before his death.

Professor Van Cleave's life was devoted to the intellectual endeavors of teaching, research, administration, editing, and active membership in numerous honorary and learned societies. With reference to the last-named it is of interest to note that he was a member of the American Association for the Advancement of Science (Vice Pres., Section F, 1949); American Society of Zoologists; American Society of Parasitologists (Vice Pres., 1935; Pres., 1947); Helminthological Society of Washington; American Microscopical Society (Pres., 1931); Limnological Society of America; Ecological Society of America; American Fisheries Society; Wild Life Society; American Society of Naturalists; Illinois State Academy of Science; Phi Beta Kappa; Phi Kappa Phi; Sigma Xi; Phi Sigma (Hon. Pres.); Gamma Alpha; Kappa Delta Phi; Beta Beta Kappa; and Alpha Kappa Lambda. In addition to his activities at the University of Illinois during the school year, he was associated during the summer months as indicated by the following: Member of the staff of the Illinois State Normal University, 1913–15; Assistant, U. S. Bureau of Fisheries, 1919, 1921; Field Naturalist, Roosevelt Wildlife Experiment Station, 1929–34; Member of the staff of the Cold Spring Harbor Biology Laboratory, 1936; and of the Isle of Shoals Laboratory in 1939. Departmentally, he rose from instructor in 1913 to Professor of Zoology in 1929. He became Acting Head of the Department from 1938 to 1939 and was awarded a Research Professorship in 1948.

Dr. Van Cleave's publications in research stand as mute testimony of his broad interests in zoology. He was one of a unique group of American Zoologists which the present specialization in college curricula and scientific knowledge make it almost impossible to produce. Although his primary scientific interests were in the broader fields of parasitology and invertebrate zoology, his specialty was a relatively small group of parasitic worms, the Acanthocephala, for which he provided a major portion of present knowledge concerning them. In addition to 243 publications he found time to write 80 reviews on current volumes in biology and engage in several interesting hobbies.

Dr. Van Cleave was an inspiring teacher who was frank, friendly, and one who appreciated and dispensed a good measure of wit and humor. He maintained a keen interest in his students during and after their graduate work at Illinois. Even unexpected visits by these always found a warm invitation welcoming them into his home where the status of the rest of the group was reviewed with enthusiasm. Dr. Van Cleave always became vitally interested in the younger members of his department and many times aided them in their research and publication which helped them to advance professionally. His personal warmth in the classrooms, laboratories, and halls of the University of Illinois as well as at the national meetings will be greatly missed by all who knew him.

JOHN D. MIZELLE

3. Report of the Treasurer. The Treasurer, Harry A. Charipper, presented the following report for the year 1953.

SEPTEMBER 1, 1952 – DECEMBER 15, 1953

Credits:

Cash balance from 1952	$3,912.50	
Receipts from dues	4,841.00	
Refund from Dr. S. Leonard (Ithaca meeting)	10.00	
U. S. Savings Bond M16486998F	740.00	
		$9,503.50

Debits:

Secretary:		
Ithaca Meeting	$200.50	
Boston Meeting	235.00	
Secretarial Asst.	680.00	
Express charges	23.28	
Stationery	38.48	
Postage	54.26	
Postage (W. Hess)	7.48	
Telephone charges	10.20	
		$1,249.15
Treasurer:		
Secretarial Asst.	300.00	
Postage	31.65	
Stationery	10.50	342.15
Wistar Institute		
Reprints	425.92	
Envelopes	33.93	
Postage	244.29	704.14
Reprints		398.14
Postage		237.79
New plates		15.83

Biological Abstracts	1,105.00	
American Institute of Biological Sciences	961.50	
Refund of dues	13.00	
Bank charges	.45	
	$5,027.15	
Bank balance — December 15, 1953	3,736.35	
1 U. S. Savings Bond	740.00	$9,503.50

4. *Report of the Auditing Committee:* The Auditing Committee consisting of Albert S. Gordon and Roberts Rugh presented the following reports:

December 22, 1953

I have gone over the record books and report submitted by Professor Harry A. Charipper, the Treasurer, and have found them in good order.

I recommend approval of the Treasurer's Report.

Signed:

ALBERT S. GORDON

December 22, 1953

I have today examined the records of the Treasurer, Dr. H. A. Charipper, and find them to be in order.

Signed:

ROBERTS RUGH

The above reports of the Treasurer and of the Auditing Committee were accepted and approved.

5. *Report of the Representatives of the Society on the Advisory Editorial Board of Biological Abstracts:* The following report was read by Alden B. Dawson:

Your representatives on the Advisory Editorial Board of Biological Abstracts are greatly indebted to Mr. G. Miles Conrad, Director of the Editorial Office, and Mr. H. I. Anderson, Business Manager, for data and information which make this report possible. In many instances their statements have been directly incorporated without giving specific credit as to source. At the outset we wish to record our great admiration for the manner in which the entire staff has cooperated to render increasing services to biologists and at the same time to keep expenses within or near the limits of potential income.

This is a task of considerable magnitude since a budget must be drawn up and expense allocations made almost a year in advance of the full realization of anticipated income. Continuously rising costs of operation and an ever-expanding biological literature combine to make financing of an abstracting system a hazardous operation.

Biological Abstracts is no longer a minor venture. The estimated expenses for 1953 are $211,880.00 and available income will be $196,102.00, leaving an estimated deficit of $15,778.00. Every effort has been and is being made to reduce expenses and to explore new sources of revenue. The change from letter-press printing to offset printing, made a few years ago, just about cut printing costs in half. Beginning in January 1954 the publication of sections F, G, H and J will be discontinued since they are not paying their way. This will reduce printing costs but there will also be a loss of subscription income occasioned by the disappearance of these sections.

The most critical and difficult problem of the operation is the preparation and publication of indexes. In an attempt to break the vicious circle of *more* literature, *more* abstracts, *larger* indexes and *more* delay in publication of indexes it has been decided to limit arbitrarily the number of abstracts published. Thus the plan is to publish 30,000 abstracts in 1954 rather than a potential 38,000. This will involve more selective coverage and the elimination of some topics formerly included: not a desirable but apparently a necessary step.

In addition, the professional staff has been divided into two groups, one for indexing and the other for editing. Thus indexing will not be a spare-time effort after abstracts are edited but the two processes will proceed simultaneously. Currently the editorial work on the 1951 subject index is well advanced and its publication in March is anticipated, to be followed by the publication in September of the 1952 index Another innovation, as of January 1954, will be the publication of a monthly author index in each issue. The publication of special abstract bibliographies as a by-product of the general abstracting service is also under study. The first effort along this line — a bibliography of glutathione — should be available early in January.

The contributions from Industry amounted to $11,675.00 in 1953 but it seems doubtful, with the coming changes in tax laws, that as much support will be forthcoming in 1954. Support from Biological Societies for 1953 was not large, considering the total outlay for abstracting. It amounted to $3,655.00.

The chief government support has been received through the National Science Foundation by way of a contract for $25,000 which is primarily intended to help bring the indexes to date. A payment of $10,000 has already been received; $5,000 is payable December 31, 1953; another $5,000 December 31, 1954, and the final payment ($5,000) is due after the Volume 28 Subject Index has been completed. There are no immediate prospects of additional government grants.

It was with great reluctance that the decision was made to discontinue in 1954 the distribution of the index to members of the American Society of Zoologists but with the reduction of the annual contribution from $2.00 to $1.00 per member it became obvious that money was being lost in every copy so distributed. Even with the omission of the index the arrangement is still not entirely one-sided. Each member subscribing to at least one Section may be credited with the amount contributed through the Society. Possibly the concept of the Zoologists' contribution as a subsidy for which we must have some return has been overemphasized. The American Society of Zoologists has consistently contributed to the support of Biological Abstracts throughout the years but the financial considerations may be greatly outweighed by the moral implications of that support. It makes it much

easier to approach industry and foundations for grants-in-aid if it can be stated that biologists themselves have demonstrated a willingness to support an undertaking which is designed primarily for their use.

Your representatives on the Advisory Editorial Board unanimously favor the continuation of an annual contribution of at least $1.00 per member.

VIKTOR HAMBURGER
KARL P. SCHMIDT
GERHARD FANKHAUSER
ALDEN B. DAWSON

It was voted that the Society would continue its contribution of one dollar per member to Biological Abstracts.

6. Report of the Representative of the Society on the Board of Governors of the American Institute of Biological Sciences. The following report was presented by Walter N. Hess:

Only a few years ago The American Institute of Biological Sciences was an idea in the minds of a few biologists. Today there is cooperation between the different biological societies which is proving to be beneficial to all concerned.

The activities of A.I.B.S. are many. With the great growth of A.A.A.S. it has become increasingly difficult to provide suitable accommodations for all societies at the annual meeting. Consequently, A.I.B.S. now arranges annual meetings of A.I.B.S. member groups who request that service. Four such meetings have been held under very favorable auspices in September on university campuses.

An addressograph file of all membership societies is maintained, and mailing service is available. It is the hope and belief of the officers of A.I.B.S. that this service will be used more and more.

The placement service — a liaison service between biologists and prospective employees — is growing and becoming more useful to both employee and employer.

The Handbook of Biological Data, to appear in several volumes, is now being prepared under the sponsorship of A.I.B.S. The first fascicle, "Standard Values in Blood," has already been published. Others are in preparation.

The *A.I.B.S. Bulletin* which appears quarterly serves as a medium of intersociety communication and for the discussion of problems of interest to biologists.

The bi-monthly publication, *The Bio-Sciences Newsletter,* originates in the A.I.B.S. offices. It is sent to the Committee for International Scientific Publication for translation, publication and distribution by the State Department to foreign countries.

The A.I.B.S. has been called upon to represent Biology on the Scientific Manpower Commission. Fields or major disciplines, rather than societies, are represented on that body.

Representatives of A.I.B.S. now speak for biologists in contacts with selective service. Several A.I.B.S. advisory committees covering such fields as physiology, biochemistry, biology and hydrobiology work in an advisory capacity in connection with the Office of Naval Research.

A.I.B.S. is now interested in the preparation of guidance literature in the biological sciences for the use of counselors and individuals interested in becoming biologists.

The advisability of establishing in Washington a "Business Office for Publications" is now being studied and the wishes of individual societies are now being sought.

The A.I.B.S. is now the operating agency of the Institute of Animal Resources. The chief aim is to record sources of production and supply of animal material, to set up standards, and to ensure the preservation of various genetic strains or stocks that are now available or that may be developed in the future.

The A.I.B.S. office is compiling data for the National Scientific Register concerning all biologists except those represented by the American Medical Association and the Federation of American Societies for Experimental Biology. About 40,000 questionnaires are in the process of being mailed.

A start has been made in recording bibliographies of scientists working in different fields so that they will be available for the use of later workers. The cooperation of authors in this program is being sought.

The A.I.B.S. is anxious to serve biologists in every way possible. Suggestions from both societies and individuals are not only welcome but anxiously received.

WALTER N. HESS

7. Report of the Alternate Representative of the Society in the Division of Biology and Agriculture of the National Research Council. The following report was submitted by C. Ladd Prosser who was unable to attend the meeting. In his absence the President read parts of the report and asked that all of it be included in the minutes.

As alternate representative of the American Society of Zoologists in the Division of Biology and Agriculture, National Research Council, I attended the annual meeting of the Division in Washington on April 24, 1953. This is a formal report to the American Society of Zoologists.

Much of the meeting was devoted to reports from various committees of the division, some of which are of interest to us as zoologists. A few of these deserve special mention. The reports of the Agricultural Board and the Agricultural Research Institute were given by Dr. L. A. Maynard (Cornell University) who has recently been made vice-chairman of the division; these reports indicate a very broad program of research and recommendations in such areas as animal health, animal nutrition, and plant disease. Related to this was the report of the Committee on Use and Care of Natural Resources by Dr. Paul Sears (Yale University) which stressed conservation of forest reserves. The annual report of the American Institute of Biological Sciences stressed the role of this institute in implementing policies of the N.R.C.; of particular interest to zoologists are the expansion of the placement service and the active progress on the Handbook of Biological Data, one fascicle of which is already published. The Institute of Animal Resources (Dr. Byerly, U.S.D.A.) is embarking on an ambitious program of collection of infor-

mation regarding sources, strains, and standards for all kinds of animals used in research, particularly by experimentalists; one goal is solution of the problem of transportation of animals from one state or country to another. The Committee on Developmental Biology (Dr. Paul Weiss, University of Chicago) is embarking on an ambitious plan for regional training seminars, aimed at strengthening the younger workers in the field. The Committee on Radiation Biology (Dr. Hollaender, Oak Ridge National Lab.) reports that all manuscripts of the three volume compendium on Radiation Biology have been received and that the first volume will be available by September 1, 1953. The Chemical-Biological Coordination Center (Dr. Karl Heumann) is tabulating chemical constants of virtually all known chemical compounds and is beginning to correlate chemical properties with biological activity; it is hoped that experimental zoologists may make use of the catalogues of this Center. An interesting description of the International Union of Biological Sciences was given by Dr. W. W. Atwood of the National Academy of Sciences.

The most immediate and most actively debated problems dealt with manpower, fellowship and federal department budgets. Dr. M. H. Trytten indicated that selective service regulations for graduate students in biology are likely to be stiffened rather than relaxed. The fellowship situation deserves serious consideration by our society. This year a total of 256 applications for postdoctoral fellowships in biology were received by the N.S.F. and N.R.C. Of these, 100 were recommended by the committees as being highly worthy of fellowships, but because of lack of funds only 20 were awarded. The A.E.C. has withdrawn completely from the fellowship field. The U.S.P.H.S. has been ordered by the Director of the Budget to withdraw from the fellowship field and the $300,000 fellowship fund cut from the U.S.P.H.S. budget. An equivalent amount has been added to the N.S.F. fellowship budget but has been distributed over the total N.S.F. fellowship program. The net result of all this is a sharp reduction in number of available fellowships, both postdoctoral and predoctoral, in biology. The situation is even worse because the N.S.F. does not appoint alternates and this year, when 18 refused fellowships, these were lost. From the number recommended by the fellowship committees, it is apparent that many more highly qualified young biologists desire fellowship training than can receive it. In a report on Fullbright fellowships, Drs. Lapp and Young pointed out that administration of the act is endangered by a recent congressional amendment stipulating that foreign funds designated for U. S. use must be assigned specifically by Congress.

A second serious budgetary problem comes in the reduction in research funds of government agencies. Zoologists, particularly in the Fish and Wildlife Service and U. S. Public Health Service are likely to have to curtail long-range programs of importance to the public welfare. The subservience of scientific agencies to politics is having very deleterious effect on morale in government laboratories. The N.R.C. took the lead in forcing an objective reconsideration of the forced resignation of Dr. Astin. General concern was expressed that important biological research will suffer severely in budget cuts. The grants-in-aid programs of the various agencies supporting much university research are seriously threatened.

Dr. Weiss, as chairman of the Division of Biology and Agriculture, pointed out that the N.R.C. cannot lobby for scientists but can present the facts to the society representatives to use as they please. Budgetary cuts in fellowships and in bio-

logical research programs directly affect us as zoologists; they can occur for political reasons or because of ignorance and prejudice. It would seem to your representative unwise for the American Society of Zoologists to send resolutions directly to congressional committees. However, I do believe that the council of the society should apprise the membership of such of the above facts as seem appropriate so that the members can act as informed citizens in expressing personal views to congressmen and the President. The specific desiderata appear to be the following: (1) Adoption of a policy of appointments of fellowship alternates by the National Science Foundation. (2) Increase in the N.S.F. fellowship fund in biological and medical sciences by an amount equal to that formerly granted U.S.P.H.S. for this purpose. (3) Objective evaluations and non-political status of scientists in government laboratories, and (4) Budget reductions in unnecessary bureaucratic frills rather than in established long-range research programs and in grants-in-aid programs.

It is my impression that the officers of the Division of Biology and Agriculture are serving us well in Washington and that they would welcome more support from us as individual scientists. Full reports of the various committees are available to any of our society members who desire them.

C. LADD PROSSER

Note appended January, 1954: As pointed out by Dr. Weiss at the meeting of the Society some of the problems presented in this report have been partially solved and the budgetary situation is eased in some governmental agencies. However, continued watchfulness is required to protect the needs of basic research.

8. *Report of the Committee on Motion Pictures.* The report was received too late to be used at the meeting, but is recorded here because it will be of interest to some of the members. Ralph Buchsbaum, Chairman, reported for the Committee:

The Committee on Motion Pictures has agreed to ask for retirement having completed its work. The original assignment was to do something about the poor quality of films being offered at the Annual Meeting of the Society.

We believe that the existence of the Committee, together with advances in quality of materials and techniques more generally available, has resulted in a superior type of product in the way of scientific films being offered to the Society meeting. This has been especially true in the past three years. The films shown at the Boston meeting were all of high professional quality.

Under these circumstances we believe that the Committee has served its purpose and can be retired. The only dissent was that of Dr. Farris who believes that the Committee should take on another responsibility, namely that of serving to advise on a film repository for scientific films. The remainder of the Committee feels that the expense of such a project is too great for our Society to undertake. Further details in this regard can be supplied.

RALPH BUCHSBAUM

9. Report of the Representative of the Society on the Board of Trustees of the American Type Culture Collection. The following report was submitted by R. P. Hall:

With the help of contributions from the various supporting organizations, the ATCC has continued to maintain a reasonably sound financial condition. A grant of $11,000 from the National Science Foundation will make possible, next year, the establishment of a collection of bacteriophages and the investigation of methods for preserving them.

For the past several years the Board of Trustees has been considering the general problems of insuring future financial security for the ATCC and obtaining some relief from the handicaps of cramped quarters and somewhat inadequate facilities for proper maintenance of cultures. The Board eventually reached the conclusion that the best available solution of these various problems would involve affiliation with the Smithsonian Institution. Accordingly, steps have been taken to interest the Smithsonian Institution in such a proposition. As a possible means of furthering this aim, Dr. Lamanna (Chairman of the Board of Trustees) has asked the Society of American Bacteriologists to act upon the attached resolution. He has requested also that the American Society of Zoologists consider the passage of a similar resolution.

R. P. HALL

The Society passed the following resolution which is similar to the one suggested by Dr. Lamanna:

The American Type Culture Collection which is sponsored by the National Research Council, the American Society of Zoologists, and other scientific societies maintains a collection of many bacteria, fungi, viruses, and other microbes. Experience has indicated that a national collection of microorganisms best serving the interests of science, medicine, education, and industry can evolve only by affiliation of such an organization as the American Type Culture Collection with some older museum type of institution. Believing that maintenance of a national collection of microorganisms is a proper responsibility of government and the community at large, as is the support of museums, zoological gardens and herbaria, be it resolved that the American Society of Zoologists lend its support to efforts seeking the development of the American Type Culture Collection into a truly national collection of microorganisms.

Furthermore, be it resolved that the officers of the American Society of Zoologists communicate the above resolution to the Board of Trustees of the American Type Culture Collection,

and that the officers of the Society be empowered to write to the members of the Board of Regents and the secretary of the Smithsonian Institution asking that earnest consideration be given to the desirability and practicality of affiliation of the American Type Culture Collection with the Smithsonian Institution.

10. Report of the Executive Committee.

(a) It was announced that the next meeting of the Society would be held during Christmas week, probably December 27, 28 and 29 on the campus of the University of North Carolina at Chapel Hill.

(b) *Society for Medical Research.* It was recommended that the Society contribute 50 dollars to the National Society for Medical Research to help it in its drive in combating anti-vivisectionist activities. A motion to this effect was passed.

(c) *Election of New Members.* In accordance with the stipulations in the Constitution regarding membership, the Committee recommended the following candidates for election to membership in the Society:

NEW ACTIVE MEMBERS

(Formerly Associate Members)

Alfert, Max	Horn, Edward C.	Mayer, William Vernon
Clark, Hugh	Keister, Margaret Louise	Orsini, Margaret Ward
Daniels, Edward William	Knobil, Ernest	Reyer, Randall William
Gatz, Arthur J.	Lehman, Harvey Eugene	Wright, Margaret R.

(Not Formerly Associate Members)

Austin, Mary L.	Holtzer, Howard	Smith, Douglas Edwin
Boss, Willis Robert	Hsu, Tao-Chiuh	Talbot, Mary
Clement, Anthony Calhoun	Jakowska, Sophie	Walsh, Michael P.
Evans, Frederick Read	Levine, Norman Dion	Warner, Francis James
Feldmesser, Julius	Miller, Faith Stone	Willis, Edwin Roy
Franzen, Dorothea S.	Nanney, David Ledbetter	Wood, Horace Elmer
Hastings, John Woodland	Rasquin, Priscilla	Wotton, Robert Moore
Holtfreter, Johannes F. C.	Ronkin, Raphael R.	Zorzoli, Anita

NEW ASSOCIATE MEMBERS

Allen, M. Jean
Bastian, James W.
Bieber, Samuel
Bliss, Dorothy E.
Bouchard, John Louis
Chalkley, Donald Thomas
Chovnick, Arthur
Foster, Morris
Foulkes, Robert H.
Fowler, Ira
Fraser, Ronald C.
Grant, William Chase, Jr.
Greulich, Richard Curtice
Grunt, Jerome Alvin
Guyselman, John Bruce
Hay, Elizabeth Dexter
Heath, Harrison Duane
Huff, George C.
Hunter, Norvell Witherspoon
King, Robert C.
Ladman, Aaron J.
Landau, Joseph Victor
Mateyko, Gladys M.
McMurray, Virginia Marion
Miller, Dwight D.
Nace, Paul Foley
Neher, George Martin
Olsen, Albert Gjerding
Piliero, Sam Joseph
Reddick, Mary Logan
Rosenberg, Evelyn Kivy
Schjeide, Ole Arne
Schneiderman, Howard Allen
Siegel, Richard W.
Smith, Thomas Charles
Sokal, Robert Reuven
Strand, Fleur Lillian
Strecker, Robert Louis
Telfer, William H.
Van der Kloot, William George
Volpe, E. Peter

It was voted that all candidates be elected as recommended by the Executive Committee, and the Secretary was instructed to cast one ballot in their favor.

11. Election of Officers: The Nominating Committee for 1953, composed of Albert Tyler, Chairman; Myron Gordon and Florence Moog, presented the following slate of nominees:

1. President, 1 year: J. Walter Wilson.
2. Vice-President, 1 year: Frank A. Brown, Jr.
3. Treasurer, 3 years: Theodore L. Jahn.
4. Executive Committeeman, 5 years: E. Newton Harvey.
5. Society Representative to the National Research Council to serve until July 1, 1956: John A. Moore.
6. Representative of the Society on the Council of the American Association for the Advancement of Science, 3 years: Harold H. Plough.
7. Society Representative on the Advisory Editorial Board of Biological Abstracts, 4 years: Edgar A. Zwilling.
8. Society Representative on the Executive Committee of the Biological Stain Commission, 1 year: Frank H. J. Figge.
9. Society Representative on the Board of Trustees of The American Type Culture Collection, 1 year: R. P. Hall.

10. Members of the Editorial Board of the Journal of Morphology, 2 years: Berwind P. Kaufmann; 3 years: E. R. Becker, Perry W. Gilbert, Libbie H. Hyman.

It was voted that the above named candidates for office be elected and the Secretary was instructed to cast one ballot in their favor.

12. Late papers. Four papers were received too late to be included in the published program. It was voted that the papers be presented and the abstracts published. The abstracts follow.

BIRD HABITATS IN THE WHITE SPRUCE-MIXED FOREST OF INTERIOR ALASKA*. William H. Drury, Jr., Harvard University. (Introduced by the Secretary.)

The basis of habitat selection by birds in the narrow zone of white spruce-mixed forest on the natural levees of the Nixon Fork of the Takotna River in interior Alaska was observed to be the life forms of the vegetation rather than species identity (floristics) Furthermore, habitat selection was observed to be based on the details of life forms and minor physiographic features of small individual areas.

The physiographic and vegetational development of the lowlands that produce the narrow zone of white spruce-mixed forest is discussed to illustrate that the establishment and survival of plant species and associations depend similarly on small-scale features of site and microclimate.

Recent works have assigned bird populations to biomes or life zones that were established on the basis of large vegetational associations. These assignments should be recognized as systems of convenience for classification. Such general classifications are not related to factors that are actively selected by birds, but instead they represent coincidences of occurrence of animals and plants that happen to live in the same area. Habitat selection is based on the details of a small site rather than on large regional types of vegetation or topography.

* Publication authorized by the Director, U. S. Geological Survey.

DIFFUSION OF NON-ELECTROLYTES AS A TAXONOMIC PROCEDURE. M. W. Fleck, University of New Mexico. (Introduced by the Secretary.)

The main facts concerning the permeability of the erythrocyte to non-electrolytes have long been known. One method of measuring permeability is the hemolysis method. This method is valuable because turbidity changes may be followed with great accuracy without instrumental aid, and it can be adapted to experiments in which the total duration of the experiment is only a few seconds.

Using the hemolysis method this investigator studied the time required for 0.3 M solutions of certain non-electrolytes to bring about 75% hemolysis in the erythrocytes of various members of the *Rodentia*. The results indicate that very

great differences in permeability may co-exist with a high degree of morphological similarity. Using a specially designed kymographic apparatus it was possible to graph the rate of diffusion. A study of the graphic records indicate that not only wide differences in permeability may exist between cells from different generic groups, but the character and properties of the curve is different in each case.

The results are of interest, therefore, from the point of view of the systematic zoologist, and may suggest possibilities for attack upon controverted questions of cell permeability by methods of comparative physiology.

RELATIONSHIP BETWEEN HEREDITARY PITUITARY DWARFISM AND THE FORMATION OF MULTIPLE DESOXYRIBOSE NUCLEIC ACID CLASSES IN MICE. Cecilie Leuchtenberger, H. F. Helwig-Larsen* and Lydia Murmanis, Institute of Pathology, Western Reserve University.

Quantitative microspectrophotometric measurements of desoxyribose nucleic acid (DNA) of approximately 1300 nuclei of various organs of mice reveal the typical basic diploid DNA value for all the somatic cells in organs of normal, pituitary recessive dwarfs and treated dwarfs. The spermatids in these mice showed the haploid DNA value for all animals whether normal, dwarf or treated dwarf. However, the microspectrophotometric measurements of DNA in mice with hereditary recessive anterior pituitary hypoplasia showed a lack of the multiple DNA classes usually encountered in normal mice. Treatment with anterior pituitary growth hormone (phyol) of the dwarf mice induced the formation of the multiple DNA classes similar to those occurring in normal control mice. These results correlate very well with the previous determination of nuclear volumes made by one of us.[1]

* University Institute for Human Genetics, Copenhagen, Denmark.
[1] Helwig-Larsen, H. F. Nuclear class series. Munksgaard, Copenhagen, Denmark, 1952.

INHIBITION OF NERVOUS SYSTEM DEVELOPMENT IN THE FROG PRODUCED BY TISSUE FRACTIONS OF ADULT HOMOLOGOUS ORGAN.[1] John R. Shaver, University of Missouri. (Lantern; 15 min.)

It has been suggested (Rose, Am. Nat., *86*, '52) that the suppression of differentiation of particular tissues in embryos by culturing them in the presence of adult homologous tissue can be explained by initial rate differences in the production of tissue-specific substances and a hierarchy of self-limiting reactions. If it is further supposed that differentiation of tissues involves the elaboration of specific cytoplasmic particle types, it should be possible to inhibit differentiation by culturing embryos in the presence of cytoplasmic particles from adult homologous organs. Repetition and extension of Roses's experiments, following the same conditions as closely as possible (sterile conditions, use of chloromycetin, culturing at 10–12°C.), corroborate his findings with adult whole tissue, and further indicate that fractions of tissue homogenates separated from cellular debris and nuclear material (cytoplasmic granules and supernatant fluid) can duplicate the inhibitory effect of whole tissue. During all stages of neurulation in all the experiments performed, cytoplasmic granules were more effective than or as effective as whole

tissue in inhibiting development of the neural system 70% of the time. In addition to delay in formation of the nervous system, various defects were observed due, in early stages, more to cellular debris and "extract" fractions than to granules or supernatant fluids. Microscopic examination of delayed and defective embryos revealed *spina bifida*, cell loss from neural groove, truncation, hypo- and hypertrophy and malformation of specific parts to be the main types of abnormality.

[1] Aided by a grant from the U. S. Public Health Service.

13. Vote of Thanks. The Society voted an expression of thanks to the Biology Department of the Massachusetts Institute of Technology for its kindness and hospitality in furnishing rooms and services for some of the sessions, to the Local Committee composed of Richard C. Sanborn, Chairman; Irwin W. Sizer and Carroll M. Williams and to the AAAS for arranging and conducting pleasant and stimulating meetings.

The meeting adjourned at 5:45 P.M.

S. MERYL ROSE,
Secretary

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(* indicates life members of the Society; † indicates charter members of the American Morphological Society; mail addresses are in italics.)

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Totals

Life members	2
Active members	987
Associate members	340
Grand total	1329

VISUALIZATION OF THE PURKINJE NETWORK OF THE BEEF HEART[1]

R. C. TRUEX, J. L. CURRY AND M. Q. SMYTHE
Departments of Anatomy and Radiology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania

SEVEN FIGURES

The left and right branches of the bundle of His and their terminal ramifications within the interventricular septum and ventricles of the beef heart have been demonstrated by numerous investigators. A variety of colloidal suspensions have been injected into the space which surrounds the large and small fascicles of Purkinje fibers as they form a subendocardial network within the left and right ventricles. The most widely used injection mass was diluted India ink for its fine particles remained in suspension and flowed readily through even the smallest spaces. Hearts prepared by this method provide good teaching specimens and were illustrated beautifully in the detailed studies of Cardwell and Abramson ('31) and Abramson and Margolin ('36). The reader is referred to the above authors for a review of the pertinent literature and a complete description of the endocardial network as observed in specimens injected with India ink.

The present study was an attempt to demonstrate this unique system of cardiac fibers by a radiographic method. The studies of Cardwell and Abramson ('31) and Abramson and Margolin ('36) had demonstrated that the injection mass often extended along the Purkinje fibers as they left the endocardium to penetrate into the myocardium. In some speci-

[1] This investigation supported (in part) by U. S. Public Health Grant H-1591.

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mens they noted that branches of the left bundle could be filled following the injection of the terminal branches of the right bundle and *vice versa*. They believed such anastomoses traversed the septum for the proximal portions of the left and right bundles contained no injection material. We hoped to demonstrate these finer myocardial ramifications of the Purkinje network by the injection of a suitable radio-opaque substance and subsequent exposure to radiographic technic.

MATERIAL AND METHODS

A total of 48 uncut adult beef hearts obtained from the abattoir were stored in a refrigerator 24 hours prior to use. The chilled hearts were always placed in warm water for a few minutes before they were opened and injection was attempted.

Several excellent specimens resulted from the injection of India ink. Hearts also were injected with different concentrations of thorium dioxide, barium bromide, barium iodide, sodium iodide, Diodrast [2] and a suspension of barium sulfate in Diodrast. The best x-ray films were obtained from hearts injected with the latter substances (i.e. 25 gm barium sulfate suspended in 50 cm^3 of 70% Diodrast mixed thoroughly for 10 minutes in a high speed blender). A warmed syringe and a No. 25 hypodermic needle were essential in order to prevent the early precipitation of the barium sulfate.

After injection the opened hearts were placed flat on the x-ray film holder and exposed by a modified extremity technic (i.e. 35–40 Kv; 1/10 sec. on 100 MA at a distance of 36 inches). Tissue blocks were taken from the injected areas of the interventricular septum and both ventricles. Following fixation and paraffin embedding, serial sections of these areas were stained with hematoxylin and eosin, Van Gieson's and Mallory's connective tissue, or Weigert's elastic technics. Foot's silver method was used to demonstrate the reticular

[2] Diodrast — A registered trade name of Winthrop-Stearns Inc., New York, New York.

fibers. Microscopic slides were made also of the dissected common crus and its branches.

RESULTS

Specimens injected with diluted India ink (1:25) were easy to prepare and the Purkinje network within both ventricles was well demonstrated after three or 4 injections. A study of these preparations corroborated the detailed report of Cardwell and Abramson ('31). We also were able to observe the injection material 8 mm beneath the endocardium and within the myocardium of the septum and ventricles. In only one specimen did we observe a filling of the rami of the left bundle branch while injecting into a distal network of the right bundle branch.

Formaldehyde fixation bleaches the myocardium and the injected black network is then prominent against a white background. Such hearts can be preserved as teaching material for long periods of time, and can be used as a source for microscopic preparations if desired.

The inorganic chemicals (i.e. barium bromide and iodide; sodium iodide and thorium dioxide) had been selected because of their molecular weights, solubilities and radiographic properties. However, we did not have a single satisfactory specimen after repeated experiments with these injection substances. They flowed readily from the needle, but seeped through the connective tissue sheath and discolored the adjacent endocardium. These chemicals were not only skin irritants, but lacked sufficient density in the small amounts used, to produce good x-ray films.

Although barium sulfate possesses a low solubility in water and alcohol, we found that small particles would remain in suspension in heated Diodrast for longer periods than at room temperature. This injection mass flowed readily through a small caliber needle and resulted in sharper x-ray films as shown in figure 1. After injection was completed this heart was closed and the radiographic film made. In this figure one can observe clearly the relationships of the left bun-

dle branch (LBB), and right bundle branch (RBB) to the ventricles (LV, RV) and interventricular septum (IS).

The gross and radiologic appearances of the right and left bundle branches (figs. 2, 3, 4, 5) are more dramatic and instructive. As in India ink preparations one is amazed by the fine network of Purkinje elements about the base of the papillary muscles in the right (figs. 2, 3) and left ventricles (figs. 4, 5). Some of the finer ramifications of this endocardial network often remain unfilled. For example, we could

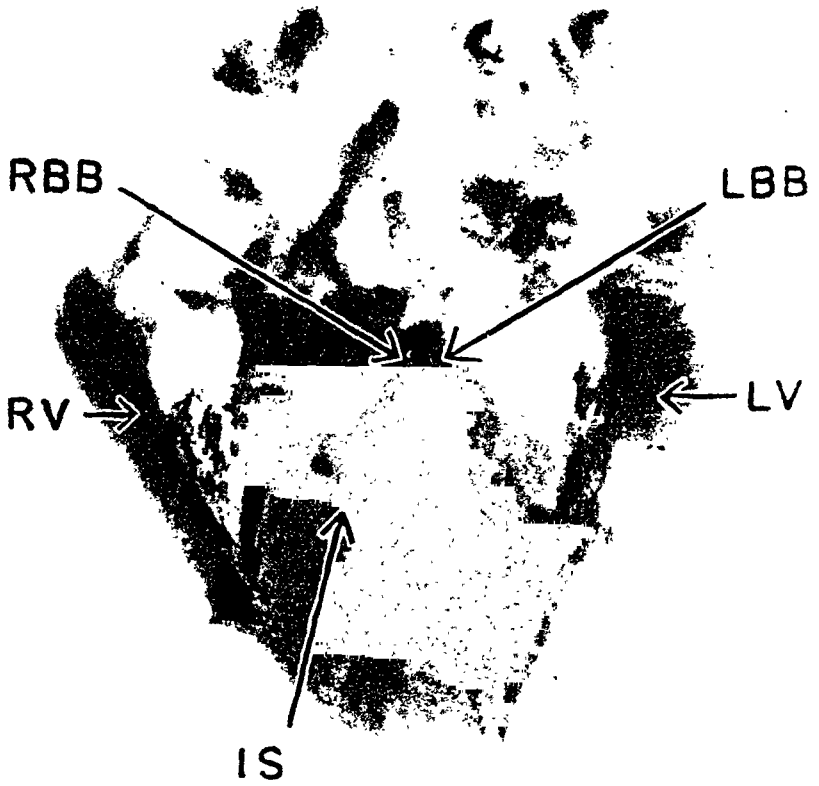


Fig. 1 Radiographic film of injected beef heart demonstrating the intact relationships of the left (LBB) and right (RBB) branches of the common bundle of His within the interventricular septum (IS), left (LV) and right (RV) ventricles.

see macroscopically the lacy network at the junction of the right ventricular wall and interventricular septum (below the moderator band in figs. 2 and 3), but these spaces defied our injection attempts. In no single specimen were we able to inject the barium sulfate suspension into every fine branch which was visible on the endocardial surfaces of both ventricles. We were disappointed particularly by our inability to demonstrate the fine ramifications of this network within the myocardium. The small peri-Purkinje-fiber space limited the amount of injection mass, so that insufficient densities prevented clear radiographic films.

Histologic sections demonstrated the injection mass within the thin connective tissue sheath which surrounds the Purkinje fibers (fig. 6). The space about several Purkinje fibers was larger than that which surrounded a few, or a single Purkinje fiber. The sheaths and spaces of the endocardial network were continuous and afforded free egress to the injection mass if the needle tip was properly placed. However, the thin connective tissue sheath was more closely related to the strands of Purkinje fibers as they entered the myocardium so that the potential injection space was reduced or absent (fig. 7). Distally the sheath loses its identity by becoming continuous with the supporting connective tissues of the myocardium.

It is interesting to note that we could not inject the common crus of the bundle of His. The reason was obvious after study of this area in injected specimens. Although there were some variations in the proximal extent of the connective tissue sheath and its subjacent space, both were absent in all specimens in the proximal three-fourths of the common bundle. As in the distal myocardium of the septum and ventricles, the sheath becomes continuous with the connective tissue stroma of the Purkinje elements and cardiac muscle.

With the use of differential connective tissue stains the wall of the sheath was found to consist of an interwoven admixture of fine elastic and reticular fibers which may have

a discontinuous inner layer of small elongated connective tissue cells. The outer wall of the sheath consists of larger elastic and collagenous fibers which are arranged parallel to the longitudinal plane of the ensheathed Purkinje fibers.

COMMENT

The teaching films from such preparations (fig. 1) were most useful in conveying to the student the intact relationships of the bundle of His and its branches. The student can also better visualize the oblique position of the muscular interventricular septum of the heart.

We believe this method offers more permanent and useful specimens than do those which are injected with fluorescent dyes and visualized by ultraviolet light (Radice, Lloveras, and Kneiszl, '48). The method may prove useful to others who desire an injection substance with adequate x-ray density for use in small vessels. The ingredients of the injection material are inexpensive, procured easily, and are relatively non-toxic.

In each of the common bundle areas we observed an enormous number of nerve fibers. Also in two cases we observed discrete ganglion cells in the connective tissue between the Purkinje fibers in this area. The functional significance of neural elements, observed previously by Truex and Copenhaver ('47), are now being investigated in this laboratory.

CONCLUSIONS

Barium sulfate suspended in Diodrast was injected into the space that surrounds the Purkinje network in the endocardium of the beef heart. The left and right branches of the bundle of His and their finer fascicles were well visualized on films by the use of a modified radiographic extremity technic. The common bundle and the fine myocardial terminals of this system were not visualized. The peri-Purkinje-fiber sheath is absent in the proximal portion of the common bundle and the small caliber of the space about the Purkinje fibers within the myocardium limited the flow of

the suspension used. The histologic structure of the connective tissue sheath and the advantages of the method are presented.

LITERATURE CITED

ABRAMSON, D. I., AND S. MARGOLIN 1936 A Purkinje conduction network in the myocardium of the mammalian ventricles. J. Anat., *70:* 251–259.

CARDWELL, J. C., AND D. I. ABRAMSON 1931 The atrioventricular conduction system of the beef heart. Am. J. Anat., *49:* 167–192.

RADICE, J. C., J. H. LLOVERAS AND J. J. KNEISZL 1948 Visualizacinones de las terminaciones del fasciculo de His por medio de la fluoresceina. Arch. Soc. Argent. Anat. N.y. Pat., *10:* 172–175.

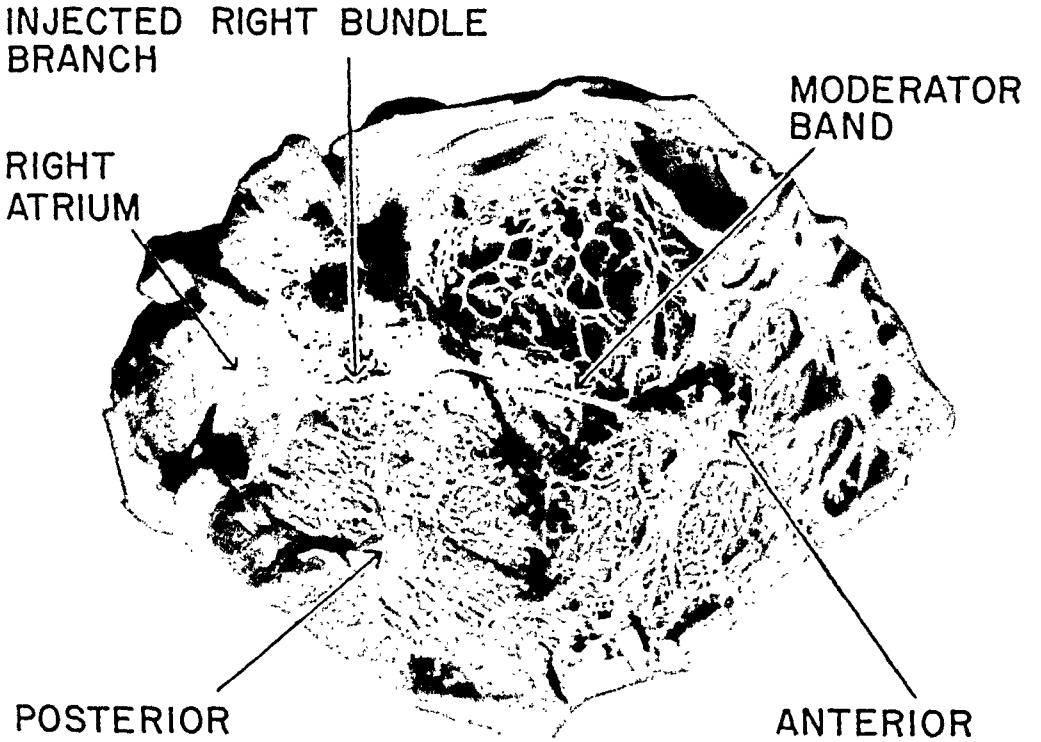
TRUEX, R. C., AND W. M. COPENHAVER 1947 Histology of the moderator band in man and other mammals with special reference to the conduction system. Am. J. Anat., *80:* 173–202.

PLATE 1

EXPLANATION OF FIGURES

2 Photograph of injected beef heart demonstrating the relations and distribution of the Purkinje network within the endocardium of the right ventricle.

3 Radiographic film of the specimen shown above in figure 2.


INJECTED RIGHT BUNDLE BRANCH
MODERATOR BAND
RIGHT ATRIUM
POSTERIOR PAPILLARY M.
ANTERIOR PAPILLARY M.

2.

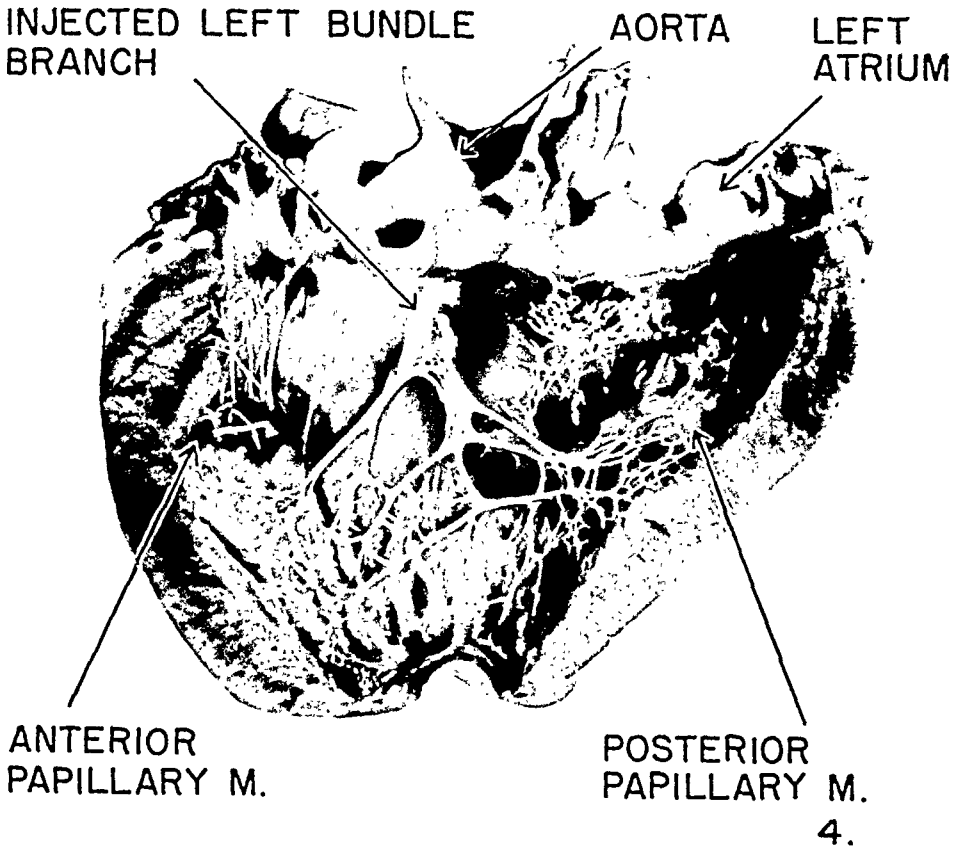
3.

PLATE 2

EXPLANATION OF FIGURES

4 Photograph of injected beef heart demonstrating the relations and distribution of the Purkinje network within the endocardium of the left ventricle.

5 Radiographic film of the specimen shown above in figure 4.


INJECTED LEFT BUNDLE BRANCH
AORTA
LEFT ATRIUM
ANTERIOR PAPILLARY M.
POSTERIOR PAPILLARY M.

4.

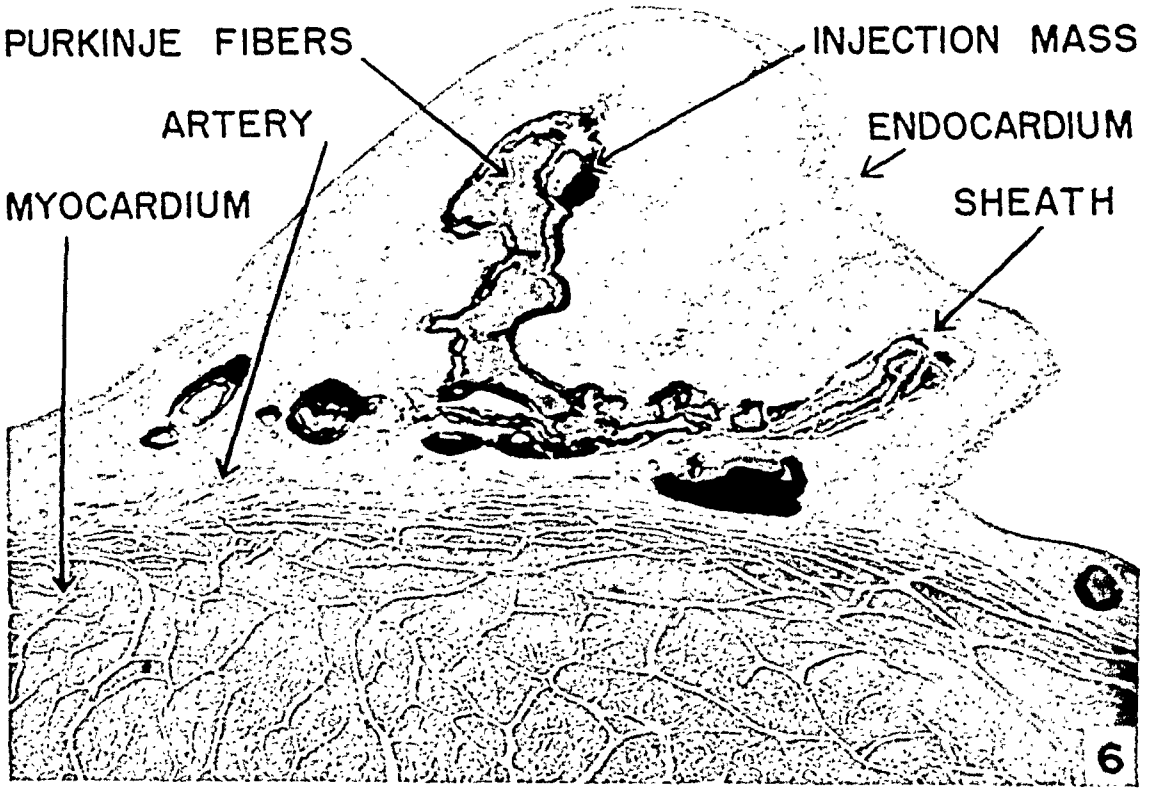
5.

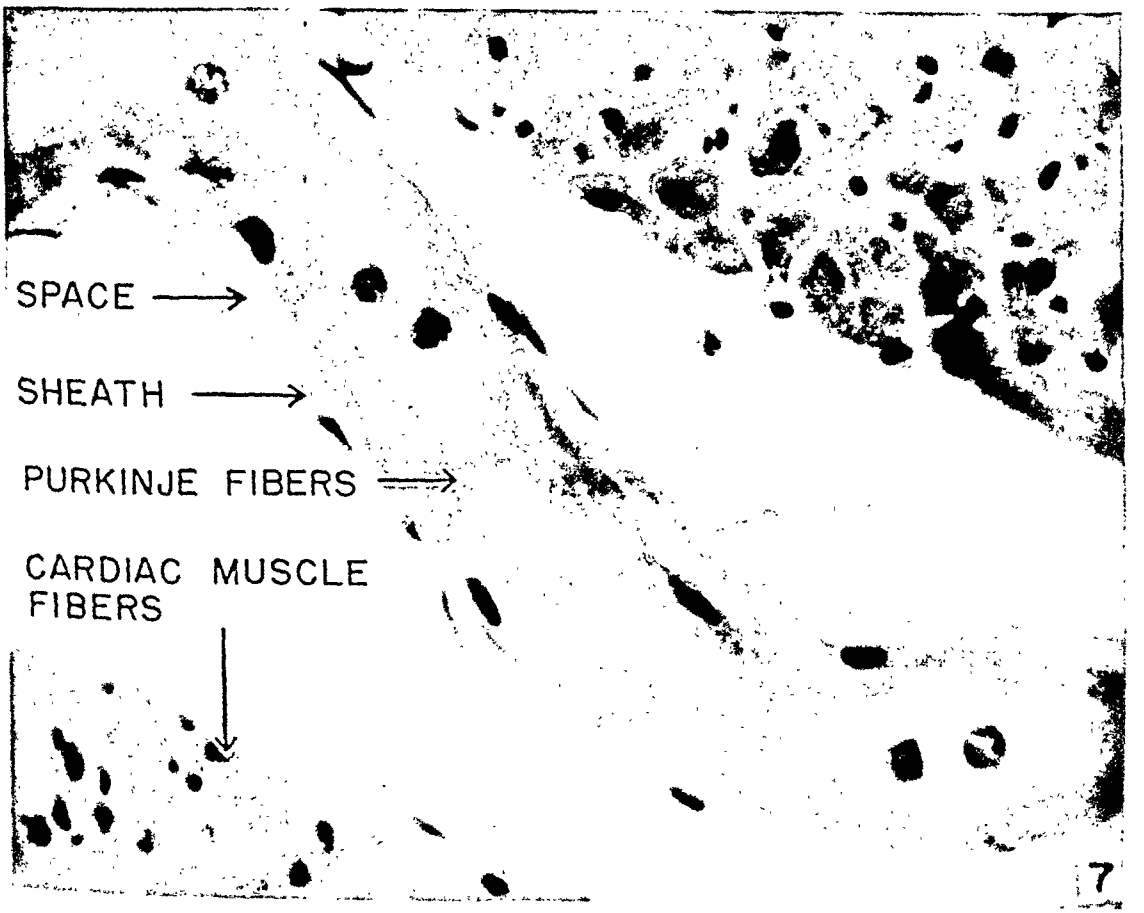
PLATE 3

EXPLANATION OF FIGURES

6 Transverse section of the interventricular septum showing the subendocardial relations of a portion of the right branch of the bundle of His. The black injection mass is observed in the space between the fine connective tissue sheath and the Purkinje fibers. Beef heart. Hematoxylin and eosin. × 100.

7 Purkinje fibers and their sheath within the myocardium of the right ventricle. The fine connective tissue sheath is closely adherent to the enclosed Purkinje fibers, so that the area of the injection space is reduced or absent within the deeper portions of the myocardium. Beef heart. Hematoxylin and eosin. × 350.


PURKINJE FIBERS
INJECTION MASS
ARTERY
ENDOCARDIUM
MYOCARDIUM
SHEATH
6


SPACE
SHEATH
PURKINJE FIBERS
CARDIAC MUSCLE FIBERS
7

A COMPARISON OF WEIGHT CHANGES IN PREGNANT AND NON-PREGNANT FEMALE RATS

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SIX FIGURES

INTRODUCTION

During the past year, in order to obtain a large number of fetal rats at various known stages of development for an embryological study, quantitative data were recorded concerning the time of copulation and other relevant conditions. As a matter of routine, the pregnant females were weighed daily. At about the same time, a similar weight check on a number of unmated females maintained on the same diet was in progress as a part of another project.

Some months later when these data were compared, the marked dissimilarity of the weight curves of these physiologically different females was noted. Donaldson ('06) graphically illustrated the normal growth curve of the rat but did not include the changes of pregnancy. Farris ('42) and others, however, have alluded to a sharp weight increase in the last days of the gestation period but no mention is made of the time of onset nor the magnitude of such increases.

It is hoped, therefore, that the present comparative study of our data will help to give these changes a more quantitative basis.

MATERIALS AND METHODS

During the last week of June, 1952 and continuing through the first week of August about 30 females were paired with males of approximately the same age and weight. Since the

weight range in the majority of animals was between 160–260 gm, 6 were discarded from consideration as lying beyond these limits. For one week all animals were placed on the same diet. During this time they were handled daily and weighed to accustom them to the laboratory routine. At the conclusion of this "breaking in" period, mating began. Starting at 5:30 P.M. E.D.T., males were placed in the cages with the females, the room was darkened as much as possible by pulling the shades and the door locked to prevent outside disturbances. At 10:30 P.M. the lights were turned on, the males removed and the females were examined. It was customary to check all cases, whether a plug was definite and unquestionable or whether a whitish granular substance only was detected around the vaginal orifice. In each instance, a small cotton swab was liberally moistened with physiological saline, gently inserted into the genital meatus, rotated between the fingers, withdrawn and lightly rubbed on a clean slide. The smears were immediately examined under the microscope. When sperm were observed, the female was isolated, her diet kept constant and her weight checked daily thereafter. It became our custom to check all suspicious cases when no sperm were found in the evening. That is, 8 to 10 hours after the previous test a new examination was made. If a positive smear was detected at this time, these too were isolated and weighed daily.

The time of copulation was arbitrarily established as midway between mating and examining time. In accordance with the method of Hall and Kaan ('42) 24 hours were allowed as fertilization time, the end of the first day then being 48 hours after "copulation time." On this basis, at appropriate periods, the female was either injected with an anesthetic dose of Nembutal and the fetuses removed under aseptic conditions or injected with a lethal dose, the embryos being taken before the death of the mother.

For purposes of comparison, the records of 25 non-pregnant females were selected whose initial weights lay within the 160–260 range. These animals were handled daily and vaginal smears were taken from time to time.

No attempt was made to control either the temperature or humidity of the animal quarters other than placing several electric fans at strategic intervals around the room.

OBSERVATIONS

In nearly 50% of the cases, the copulation plug was questionable, often lying deep in the vagina. However, since I had already decided to make smears, it was quite surprising that in many instances where a plug was most definite, sperm did not appear in the smear. More or less by chance, but later as a matter of course, these same females were re-examined 8 or 10 hours later. In each instance, sperm in large numbers were recovered. It appears that the plug, which seemed rather firm in the evening, had undergone a liquefaction, releasing many sperm previously trapped by coagulation.

Since all weight records had been kept in graphic form, it soon became apparent that the characteristic of the curve depended, to a great degree, upon the initial weight of the animal when the tests were begun. For this reason all the females were grouped into two classes. In the first class were those females whose weights ranged between 200 and 260 gm; in the second were those between 160 and 190 gm. In most cases, the graph for the normal female compares favorably with those of Donaldson ('06).

Group I

The first chart (fig. 1) indicates that a striking difference between the curves of pregnant and non-pregnant animals occurs after the 7th day. The bar graph (fig. 2), which represents the daily average gain or loss, seems to indicate an even earlier divergence, little similarity appearing after the first day.

It was further discovered from an analysis of each individual curve that the average non-pregnant female showed a change in the curve, either a static period or one of loss, every three days (fig. 3). In addition, the average normal female gained for no more than three consecutive days. In one instance, a

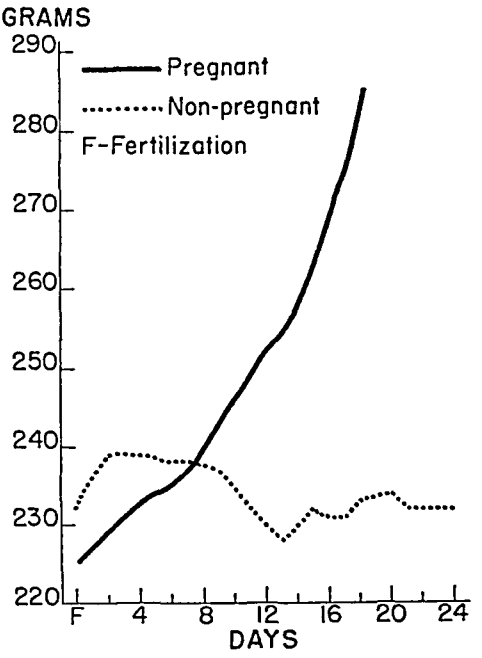

Fig. 1 Weight charts for the pregnant and non-pregnant females in group I taken as an average of the group as a whole.

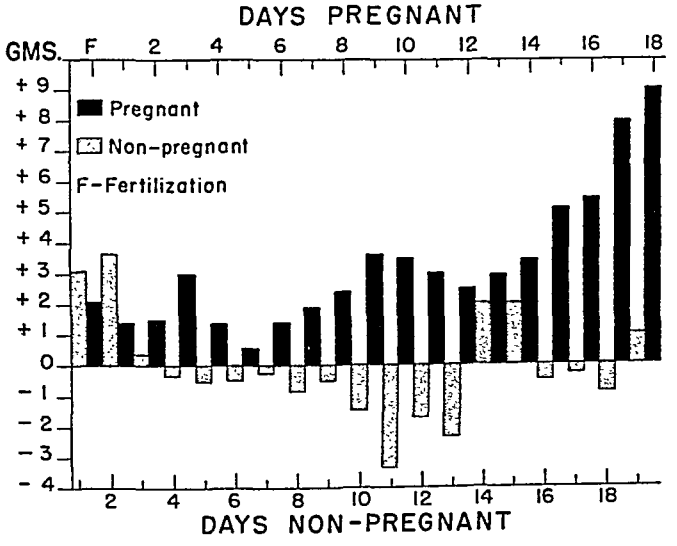

Fig. 2 The bars on this graph represent the average daily gain or loss for the group of pregnant and non-pregnant females whose initial weight ranged between 200 and 260 gm. This record covers an 18 day period, selected at random from the records of normal animals to compare with the first 18 days of pregnancy.

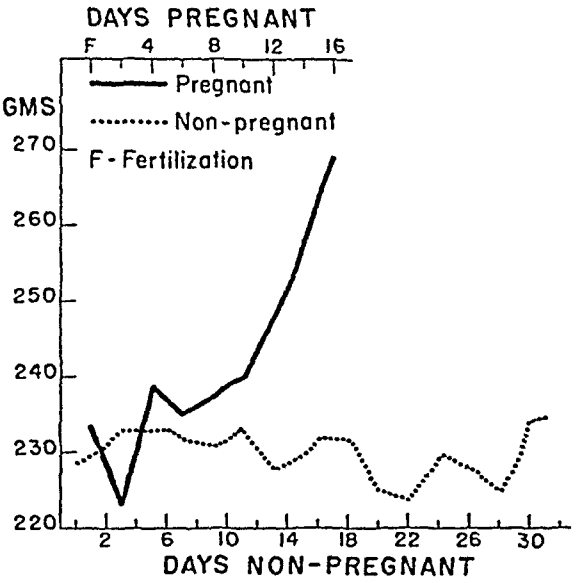

Fig. 3 This chart represents the day to day changes in two individual group I females.

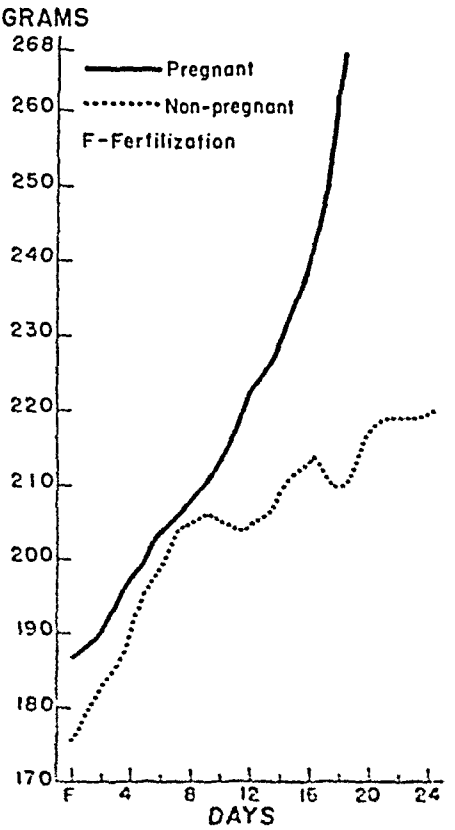

Fig. 4 Weight chart for the average pregnant and non-pregnant females in group II. The arrangement is similar to figure 1.

7 day consecutive gain period was recorded, and, in two others, 6 day periods were noted.

On the other hand, the pregnant females of this group showed directional changes in the curve on the average of every 5.5 days, and, on the whole, showed neither static period nor loss after 6.5 days (fig. 3). Three of the animals in this group showed no loss at all, while an equal number showed a very slight slacking off on the 12th day.

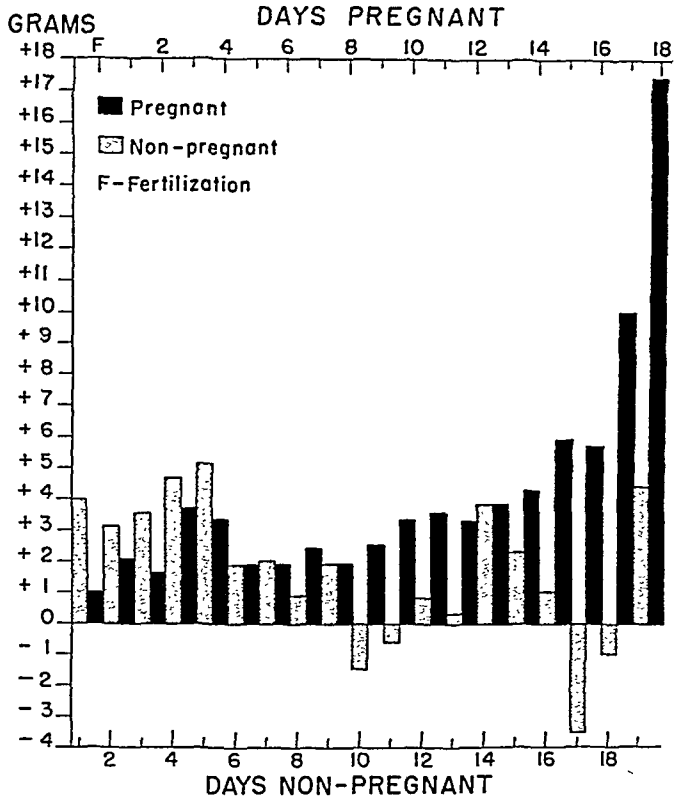

Fig. 5 The bars in this graph represent the average daily gain or loss of pregnant and non-pregnant females in group II. The arrangement is similar to figure 2.

Group II

The weight curves of pregnant and non-pregnant animals show definite similarity until the 8th day of pregnancy (fig. 4). This is also shown on the bar graph (fig. 5), the pregnant animals showing a consistent daily gain of 2.5 gm or more after the 8th day while the non-pregnant animals showed great irregularity.

In this group, too, the non-pregnant females showed changes in the curve about every three days. The pregnant animals showed a change every 7.5 days. The non-pregnant animals showed an average gain period of 3.5 days without a break (fig. 6), while the pregnants averaged about 8.5 consecutive days. In two instances, non-pregnant animals showed a gain period of 9 days. The average pregnant female showed no loss after 5.5 days (fig. 6) although one showed a slight recession on the 12th day and another had a break on the 10th day.

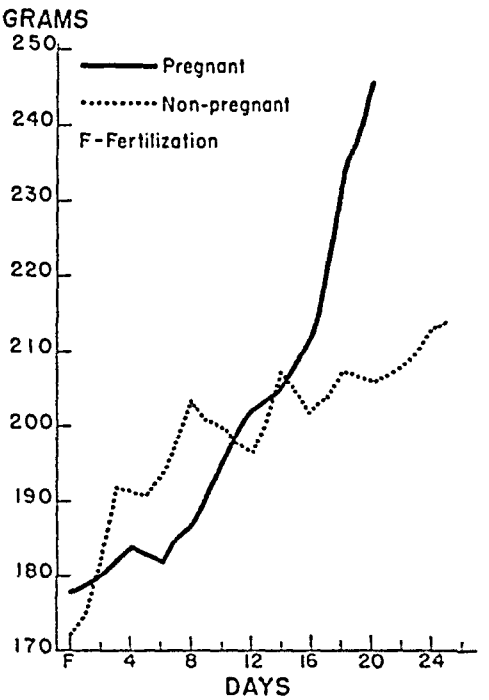

Fig. 6 This graph represents the day to day changes in two individual group II females.

SUMMARY AND CONCLUSIONS

1. An examination 8 or 10 hours after suspected copulation is more reliable for finding sperm in the vaginal smear of rats than a more immediate examination.

2. By means of a daily record of weight changes, pregnancy could be definitely established in 50% by the 7th day and in 80% by the 9th day for female rats whose initial weight was 200 gm or over.

3. In females whose initial weight was between 160 and 190 gm pregnancy could be detected in the same way in nearly all cases by the 9th day.

4. We do not believe that environment is responsible for these weight differences.

5. A study of individual graphs for each non-pregnant female reveals that fluctuations in weight are more or less rhythmic. The cause of this phenomenon and its relation to other vital processes needs further investigation.

LITERATURE CITED

DONALDSON, H. H. 1906 A comparison of the white rat with man in respect to growth of the entire body. Boas Memorial Volume. G. E. Stechert and Co., New York, pp. 5–26.

FARRIS, E. J. 1942 Breeding of the rat. The Rat in Laboratory Investigation. J. B. Lippincott Co., Philadelphia, pp. 1–5.

HALL, A. R., AND H. W. KAAN 1942 Anatomical and physiological studies on the thyroid of the albino rat. Anat. Rec., *84:* 221–239.

DEVELOPMENT OF CLEAVAGE LINE PATTERNS IN THE HUMAN FETUS

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TEN FIGURES

The existence of cleavage line patterns in the human adult is amply demonstrated, they have also been recognized in the cat (Gardner and Raybuck, '51). Upon reviewing the literature it was found that the earlier investigations have been performed on adult material. The present investigation was made in order to obtain information on (1) the period of fetal development when cleavage lines first appear and (2) the developmental stages of the fetal cleavage line patterns.

MATERIALS AND METHODS

A total of 16 fetuses and two stillborns were used. The age range was from two months (20 mm crown rump length) to birth. All of the specimens were fixed in formalin. In a previous investigation we ('51) found that formalin fixation did not alter the production or the pattern of cleavage lines. The puncture wounds in the larger specimens were produced by piercing the skin with a Mall probe that had been ground to a sharp conical point, while in the smaller specimens a sharp dissecting needle was used. The wounds were painted black and photographed. One problem encountered in painting the wounds was that the paint would flake when the fetus was returned to the specimen jar. Several types of paint were tested

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for adhering qualities. Black casein paint was found to be unaffected by the fixing fluid; in addition it has a rapid drying time.

To corroborate the gross anatomic evidence histologically, blocks of tissue containing the linear wounds were removed and sectioned in three different planes: (1) the vertical plane parallel to the long axis of the wound, (2) the vertical plane at right angle to the long axis of the wound, and (3) the horizontal

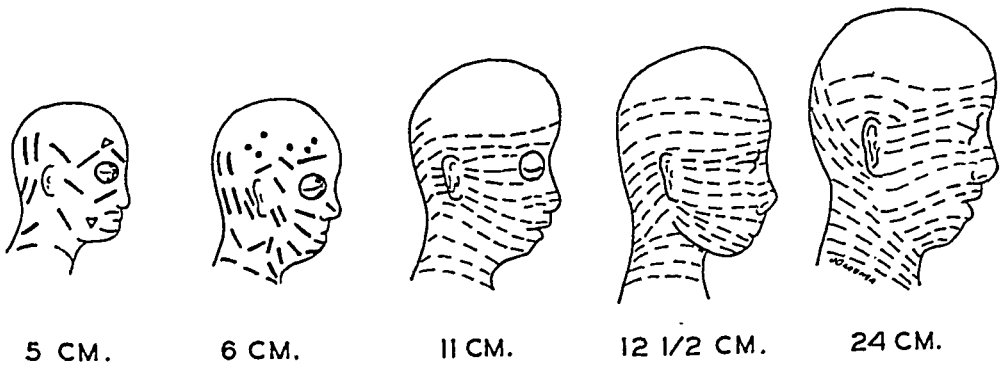


Fig. 1 Developmental alterations of the cleavage lines of the face.

plane. The sections were prepared with Masson's trichrome stain in order to study the relationship of the blue staining collagenous and the red staining elastic fibers to the long axis of the wound.

OBSERVATIONS

A definite sequence of events has been observed in the cleavage line patterns of the human fetus. The stage at which cleavage lines appear varied within narrow limits. In the CR 20 mm fetus cleavage lines were considered absent as the wounds were circular instead of linear. Two specimens of similar size presented opposite results. In a CR 28 mm fetus it was possible to produce linear wounds, however they were intermixed with circular wounds. While in a slightly larger fetus, CR 32 mm, cleavage lines were completely absent. In the 50 to 60 mm group 4 fetuses were studied. All of the specimens presented linear puncture wounds. One 60 mm fetus, however, presented scattered circular wounds on the head,

hands and feet. All of the specimens with a CR length greater than 60 mm presented linear wounds.

Comparison of the fetuses of various ages revealed that in some regions the pattern changed little as the fetus developed while in other regions marked changes occurred. The cleavage line pattern of the face begins with the long axis of the wound curving around the orbits and lips (50–60 mm stage). As development continues the long axis assumes a position parallel

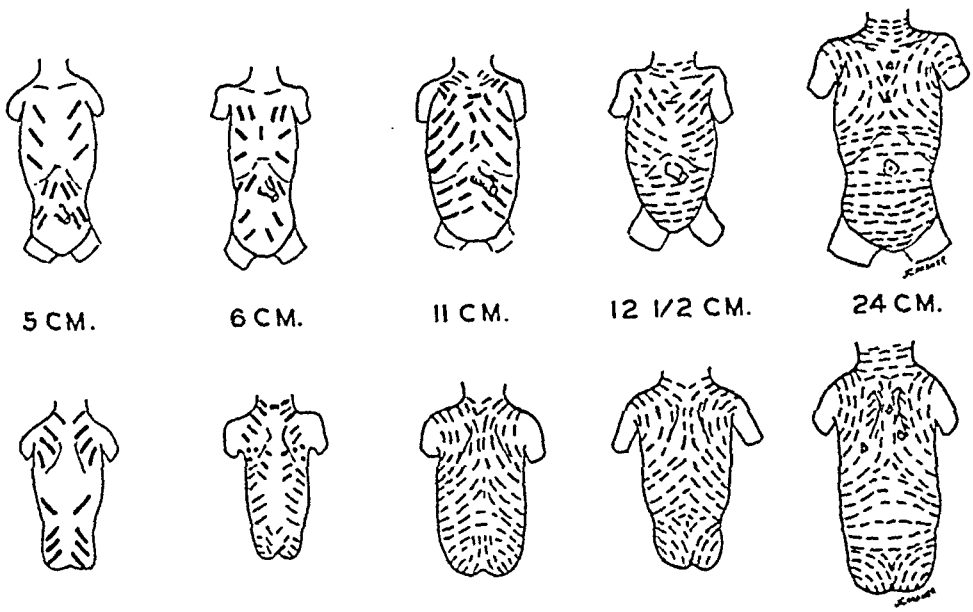


Fig. 2 Developmental alterations of the cleavage lines of the trunk. Above, anterior view; below, posterior view.

to the horizontal plane and undergo no alteration with development (figure 1). In the nucha the pattern was not consistent except in the early fetuses of 50–60 mm in which the long axis of the wound was near vertical. The patterns of the trunk presented noticeable changes. Cleavage lines of anterior thoracic wall first appear running downward and forward in the 50 mm fetus. The next step in development occurs in the 60 mm stage, in the lower thoracic region the long axis of the wound begins to rotate and by the 110 mm stage the basic thoracic pattern is established, that is, in the upper thoracic region the linear wounds run caudal and anterior while in the

lower thoracic region they run cephalic and anterior. The rotation in the lower region thus gives curved pattern with the convex side of the curve facing the midline. The posterior thoracic wall undergoes similar changes. The pattern of the anterior and posterior abdominal walls also undergo developmental changes. The linear wounds run cephalic and anterior in the 50 mm fetus, gradual rotation occurs and by birth the cleavage lines are parallel to the horizontal plane.

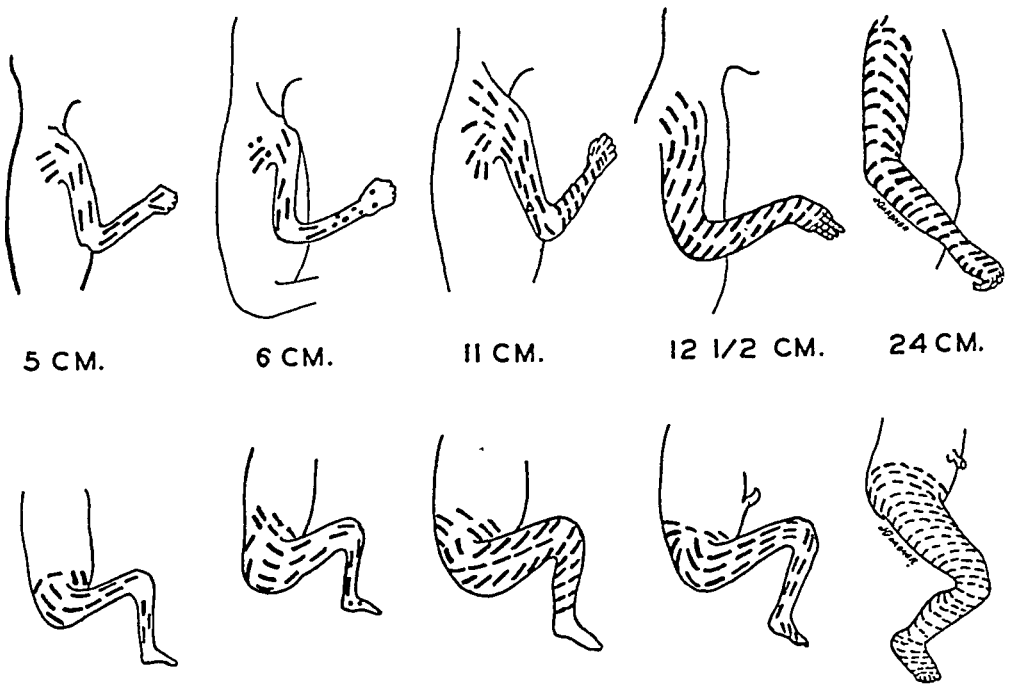


Fig. 3 Developmental alterations of the cleavage lines of the extremities. Above, superior extremity; below, inferior extremity.

The most striking pattern changes occur in the extremities and the alterations are similar in both the upper and the lower extremity. The linear wounds first appear parallel to the long axis of the extremity. In the 110 mm fetus rotation of the linear wound was first noticed and in the 240 mm stage a 90 degree rotation becomes evident. The resulting wounds are perpendicular to the long axis of the extremity, the hand or foot and the digits. The cleavage lines of the knee and elbow undergo the same alterations as the other regions of the extremity.

Stillborns have cleavage line patterns that correspond to those observed in the 240 mm fetus. Regions in which the lines of tension are diverse irregular shaped wounds appear. Such wounds, though few and scattered, were observed as early as the 50 mm stage; they are however, more noticeable in the 250 mm fetus and stillborn. Irregular shaped wounds occur, as in the adult, primarily in two regions, the sternal region and the interscapular region, occasionally they may be present in the sacral region.

The histologic preparations confirmed the earlier reports of Cox ('41) and Gardner and Raybuck ('51), in which it was noted that the linear wounds show a preponderance of collagenous and elastic fibers running parallel to the long axis of the wound. In the present investigation it was observed that the collagenous fibers began to run parallel to the long axis of the wound in the 50 mm fetus and the developmental changes are represented by an increase in the number and size of fibers.

DISCUSSION

Histology textbooks described fibroelastic connective tissue as a supporting tissue consisting of an interlacing system of collagenous and elastic fibers. Such a description leaves the reader with the idea that the tissue is merely a maze of fibers that bind structures together but is devoid of definite structural pattern. The results of earlier investigations (Dupuytren, 1834; Langer, 1861; Kocher, 1892; Cox, '41; Gardner and Raybuck, '51) describe a definite structural pattern of the fibroelastic connective tissue in the dermis and the pattern in reality represents lines of tension. The lines of tension indicate the direction in which the skin splits when it is pierced by a sharp conical instrument, thus they are referred to as cleavage lines. That they are lines of tension is illustrated by the fact that a wound inflicted by a cutting instrument at right angles to the tension lines will gape; one inflicted parallel to their long axis will remain narrow with approximated edges. The results of the present investigation indicate that collagenous fibers appear in most cases in the 50 mm fetus, however,

in some the presence of cleavage lines in the younger specimens (28 mm fetus) indicates an earlier origin of the fibers. The orderly arrangement of the linear wounds in the young specimens suggest that during the development of the collagenous fibers existing internal forces of the growing fetus determine the manner of their distribution. As the fetus grows the cleavage line patterns undergo regional alterations. Rate of

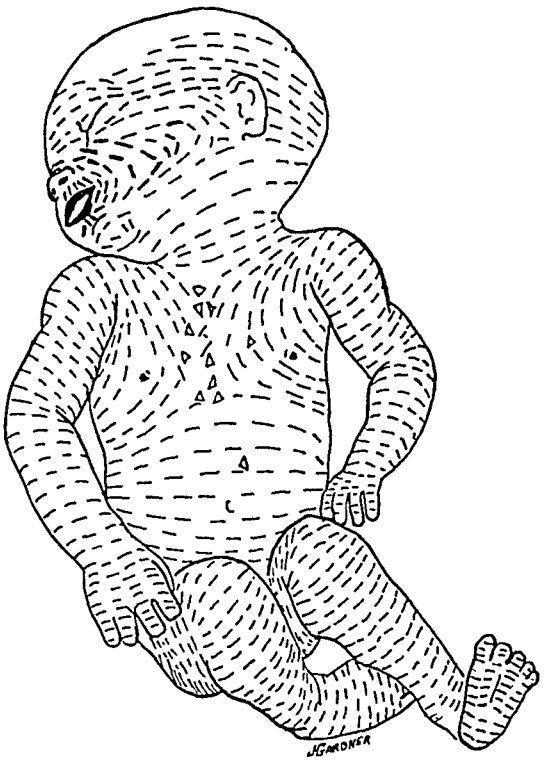


Fig. 4 Cleavage line pattern of the stillborn. Anterior view.

proportional growth of various structures in a particular region appears to be the influencing factor in regional alterations. Cleavage lines of the face first appear curving around the eyes primarily and to a lesser degree the lips; as the size of the eye decreases in proportion to the remainder of the face the cleavage lines become arranged on a horizontal plane. The pattern of the abdominal wall is similarly influenced; as the abdominal contents increase in size and the abdomen becomes

distended the cleavage lines which first appeared to run cephalic and anterior progressively rotate to a horizontal plane. Growth and development also affect the pattern of the extremities. Early growth of the extremities is primarily outward and during this period the cleavage lines are directed parallel to the long axis of the extremity. As the outward growing extremities begin to increase in circumference the cleavage lines rotate to a horizontal plane. The rotation of cleavage lines in the upper and lower extremities differ from one another by rotating in opposite directions. In the upper extremity the distal end of a wound appears to rotate in a posterior direction in order to assume the horizontal plane, while in the lower extremity the distal end of the wound appears to rotate in an anterior direction. This difference might be influenced by limb rotation, that is, the anterior lateral rotation of the upper extremity and the posterior medial rotation of the lower extremity.

Compared to the adult the cleavage lines of the newborn differ in certain regions. In general the patterns of the head, the neck, and the trunk of the adult and newborn are similar; however, differences are noted in the extremities. The adult has a more complex pattern, particularly in the palmar and the plantar regions, the region of the elbow and the knee. Data on pattern alterations occurring between birth and adulthood are wanting.

SUMMARY

The dermal fibroelastic connective tissue of the fetus is arranged in such a manner as to represent cleavage lines (lines of tension). Cleavage lines were observed as early as the 28 mm stage but in most cases they do not appear until the 50 mm stage. The various regions of the body present individual cleavage line patterns which undergo alterations as the fetus grows. The pattern alterations are influenced by (1) rate of proportional growth and (2) principal direction of growth of certain structures in the various regions. The cleavage line pattern of the newborn differs from that of the adult in certain regions.

LITERATURE CITED

COX, H. T. 1941 The cleavage lines of the skin. Brit. J. Surg., *29:* 234–240.

DUPUYTREN 1834 Traité théorique et pratique des Blessures par Armes de Guerre, 2 volumes, 522, 527 pp., J. B. Baillière, Paris.

GARDNER, J. H., AND H. E. RAYBUCK 1951 Cleavage line patterns of the cat. Anat. Rec., *100:* 549–556.

KOCHER 1892 Chirurgische Operationslehre. viii + 208 pp., G. Fischer, Jena.

LANGER, A. K. 1861 Zur Anatomie und Physiologie der Haut. Ueber die Spaltbarkeit der Cutis. Sitz. k. Akad. Wissen., Wien, 44.

PLATE 1

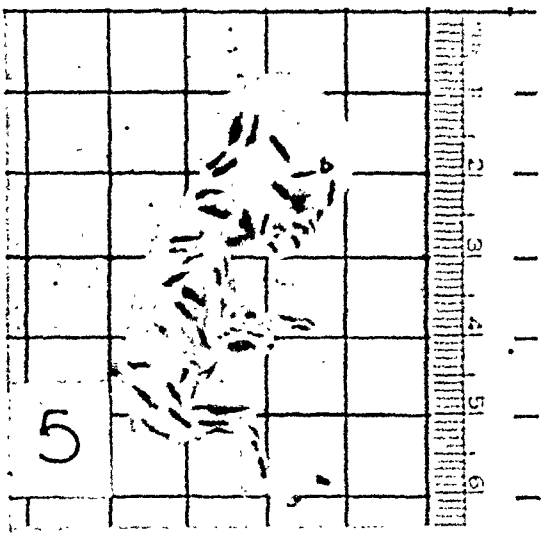
EXPLANATION OF FIGURES

5 Fetus: age 3 months, crown rump length 55 mm. Lateral view of cleavage line pattern.

6 Fetus: age 4 months, crown rump length 110 mm. Lateral view of cleavage line pattern.

7 Fetus: age 7 months, crown rump length 240 mm. Three quarter view of cleavage line pattern.

8 Posterior view of specimen in figure 3. Note triangular shaped wounds in the lower interscapular region.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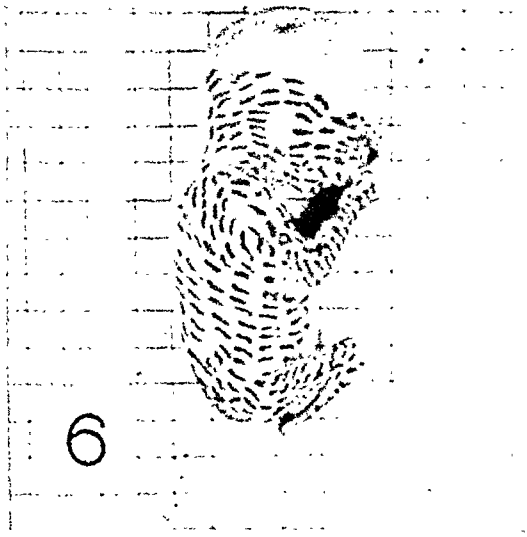
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9 Cleavage line pattern of the stillborn. Anterior view.

10 Cleavage line pattern of the stillborn. Posterior view.

AUTORADIOGRAPHIC VISUALIZATION OF S^{35} INCORPORATION AND TURNOVER BY THE MUCOUS GLANDS OF THE GASTRO-INTESTINAL TRACT AND OTHER SOFT TISSUES OF RAT AND HAMSTER

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TWELVE FIGURES

INTRODUCTION

Studies with S^{35} labeled sulfate (Dziewiatkowski, '49) have confirmed the classical concept that only a small proportion of sulfur introduced in mineral form, is retained by the organism. This author ('51a) has demonstrated in suckling rats that part of the sulfate retained was deposited in the cartilage and bones. Layton ('51) has shown intake by a variety of tissues in vitro and in vivo, with emphasis on the specific intake of healing wounds where "it is probable that sulfate ions are utilized in the synthesis of chondroitin sulphate present in the connective tissue ground substance and in the collagen." Recently, Boström and Odeblad have demonstrated by autoradiography ('53) that labeled sodium sulfate, administered to a mother, is localized in several tissues of the foetus where it is found in form of mucopolysaccharides ('53); Asbol-Hansen ('53) has reported S^{35} synthesis by mast cells in the vicinity of cancerous tissues. In this laboratory, a new program of studies with S^{35} to compare with previous work on bone and tooth growth where P^{32} and Ca^{45} were utilized (Leblond, Wilkinson, Bélanger and Robichon, '50;

Bélanger and Leblond, '50; Bélanger, '52) has also permitted the autoradiographic examination of soft tissues. Localization of the labeled sulfur in the membranes of the inner ear and specific synthesis by the mucous neck cells of the stomach have been published elsewhere (Bélanger, '53a, b). The present report is a general account and discussion on the phenomena observed in soft tissues, following a single injection of inorganic sulfur.

MATERIALS AND TECHNIQUES

Rats of 4 to 12 days of age, of the Sprague-Dawley strain and also 4 day old Hamsters were injected under the dorsal skin with a single dose of 5 μc S^{35} separated isotope, obtained as weak sulfuric acid. They were sacrificed at intervals of one hour, two hours, one day, two days and 4 days. The tissues were fixed in a mixture of three parts 95% ethanol and one part neutral formaldehyde. Following routine dehydration and paraffin or celloidin embedding, sections of 10 μ were made and affixed to glass slides. Autoradiographs were obtained by coating with photographic emulsion according to the improved inversion modification of the melted emulsion technique (Bélanger and Leblond, '46; Bélanger, '50, '52). These were exposed for periods of three to 4 days and up to three months.

A comparable study of metachromasia was carried out on sections of the same tissues. These sections were stained for three minutes with a 0.5% solution of toluidine blue in 5% ethyl alcohol and the effect of staining was first observed in distilled water without differentiation. For high power studies, the sections were mounted from water in Crown immersion oil,[1] a modified mineral oil which has no effect on the stain.

OBSERVATIONS

Following short exposures of three to 4 days, intense autoradiographic reactions were observed over the cartilage, bones

[1] Techni-Products Co., Buffalo, N. Y.

and teeth of animals injected within 24 hours. These shall be described at a later date.

Mucous glands. Amongst soft tissues, the mucous salivary glands and parts of the gastro-intestinal tract revealed a relatively large fixation of S^{35}.

The major mucous salivary glands (fig. 7) have shown a very intense overall intake of S^{35}. The mixed glands have shown the presence of radiosulfur in the mucous portion of the acini.

Some minor salivary glands of the palate have shown particularly well the entry and transit of S^{35}. One hour after the injection of tracer, the autoradiographic picture was located to the distal portion of the acinar cells. At 24 hours, the image showed penetration of the whole cells. At two days, radioactive mucus was seen in the lumen and along the excretory duct. At 4 days, there was no more evidence of radioactivity.

By comparison, there was much less radiosulfur in the tubulo-acinar glands of the olfactory mucosa but the output was apparently slower since at 4 days an autoradiograph was still recorded over the acinar portion.

Gastro-intestinal tract. In autoradiographs of short exposure, the non-glandular portion of the stomach showed no visible image. On the other hand, the mucosa of the glandular stomach (figs. 1, 2, 3, 4) gave intense reactions, with site and age differences. The proximal part of the glandular stomach (fig. 1) exhibited an abrupt reaction initiating with the change from squamous stratified to glandular epithelium. The autoradiographic picture had the appearance of parallel streaks overlying the glands of that area. The reaction was initially located to the whole length of the tubular glands in the 10 day old rat (fig. 1). After a short distance towards the pylorus, where the glands are growing in length (fig. 2), the autoradiographic picture has remained localized near the surface and has thus appeared as proportionally less extensive. Over what appears to be the area of largest

glandular development of the enzymic mucosa (fig. 2), the autoradiographic picture was minimal and localized to the portion of the gland known as the neck. The surface cells in that area as well as the enzymic portion of the gland distal to the neck did not exhibit the presence of radiosulfur.

In the pyloric portion of the stomach (figs. 3 and 4) the reaction became more generalized again, covering progressively the whole area of the pyloric glands. A difference in this extension of the autoradiographic picture was noted between rats of 4 days and rats of 10 days of age, the older ones showing a more intense and more extensive intake (fig. 4).

The intense glandular reaction ceased abruptly with the change in the appearance of the epithelium on the duodenal side of the pylorus (figs. 3 and 4). It has been replaced by the isolated punctiform pictures of mucous goblet cells which were seen to increase in number from the duodenum (figs. 3 and 4) to the ileon (fig. 5), to the colon (fig. 6) where two hours after the subcutaneous injection of tracer, some radioactive mucus was seen in the lumen of the organ. The Brünner glands of the duodenum (figs. 3 and 4) have never shown evidence of sulfur fixation.

Sections of the stomach from animals sacrificed two days after the administration of S^{35} (fig. 8) failed to demonstrate radiosulfur in the mucosa of this organ but gave strong images of mucous secretion in form of streaks mixed with the chyme or occupying a superficial position in regards to the stomach content.

With an exposure of 30–90 days of sections from animals sacrificed one to two hours after the injection of tracer, a uniform wide band appeared at all levels of the digestive tract (fig. 9) at the junction of the mucosal epithelium and the subjacent chorion, the area of the basement membrane.

The liver gave a weak overall picture with increased intensity over the wall of the larger arteries and veins. The wall of the extra-hepatic ducts and the pancreatic ducts have shown evidence also of fixed radiosulfur.

Connective tissue. Over other portions of connective tissue: tendons, aponeuroses, mesenteries, reactions of a similar order of magnitude were apparent after long exposures.

A thick portion of apparently amorphous connective tissue underlying the molar primordia in the upper jaw, showed a diffuse, strong picture.

Diffuse autoradiographs of minor intensity were also recorded over the sclera and the cornea.

Sharp pictures have also been recorded over the mast cells (fig. 10) and sometimes also over granular masses in their immediate vicinity.

These cellular localizations have remained strong in animals sacrificed up to 4 days after the introduction of tracer while no appreciable autoradiographs were recorded over other parts of the connective tissue after two days.

Blood vascular system. The walls of the blood vessels, arteries and veins (fig. 12) were positive in a ratio proportional to size.

The heart gave a relatively strong record of S^{35} over the atrio-ventricular valves and a moderate reaction along the endocardium. There was a moderate picture over the larger coronary vessels.

Lungs. The lungs showed a moderate reaction along the bronchial tree, the reaction decreased progressively towards the respiratory complex.

Uro-genital system. In the kidney and urinary tract (figs. 11 and 12), radioactivity was recorded in specific areas. The capsule was moderately active; so was the wall of the larger blood vessels. The medulla of the kidney, particularly, the sub-cortical portion and the medullary rays gave weak autoradiographs after long exposures. The inner lining of the pelvis and of the ureter appeared also as positive (fig. 12). In cross sections of the ureter, there was a ring like reaction at the border of the epithelium and the subjacent chorion.

The adrenal capsule was weakly positive.

The testis showed a moderate reaction at the border of the seminiferous tubules, and a more intense one over the tunica

albuginea of the organ. The epididymis also showed a moderate reaction bordering the epithelium, increasing in intensity towards the ductus deferens which was more strongly reactive than the epididymis. A weaker picture was recorded outside the epithelium of the urethra and also along the trabeculae of the cavernous tissue. The prostate gave a relatively strong reaction over the capsule and also along the inner trabeculae.

Lymphoid tissue. Lymphoid tissue (fig. 7) was generally negative, with the exception of the spleen which showed small scattered masses in the immediate vicinity of the sinuses, identified later as clusters of megakaryocytes, and also along the wall of the centro-lobular artery.

The thymus gave a moderately intense image over the trabeculae but was otherwise negative.

Skin and accessories. Over the papillae of the tactile hair follicles (fig. 10) and also over what appears to be the capsule outside the cavernous sinus (Melaragno and Montagna, '53), a diffuse autoradiograph was apparent.

The epidermis and the epithelial and keratinized portions of the hair did not exhibit the presence of radiosulfur.

The anal gland showed a moderate picture along the capsule and at the immediate border of the sebaceous sacs. The glandular cells and their product were negative.

Inner ear membranes. Specific and quantitatively different pictures were seen over the membrana tectoria, the crista and the otolithic membrane of the inner ear and were reported previously (Bélanger, '53a).

Metachromasia. Sections from the above mentioned tissues exhibited metachromasia in all areas demonstrating the presence of radiosulfur: cartilage, bones, dentine, dental papilla, mucous glands, goblet cells, mast cells, tendons, aponeuroses, heart valves, wall of blood vessels and major ducts, medulla of the kidney, sclera and cornea, inner ear membranes, papilla and capsule of the hair follicles (Sylven, '50; Melaragno and Montagna, '53) were positive at various degrees. The large radioactive area of connective tissue sub-

jacent to the tooth buds in the upper jaw, stained a brilliant magenta with toluidine blue. The cytoplasm of the megakaryocytes of the spleen stained also metachromatically. The mucus of the surface cells of the stomach and that of the Brünner glands of the duodenum did not stain with toluidine blue. Sections of human stomach and duodenum fixed in aqueous formaldehyde and stained according to the procedure described here, gave identical results.

DISCUSSION

It appears from these observations as well as from previous ones (Layton, '51; Boström and Odeblad, '53) that the small amount of incoming sulfate which the organism can retain, has a widespread distribution, at least in the very young animals. A large range of proportional intake has also been recorded among the tissues and organs exhibiting the presence of radiosulfate. The largest part of this material has been found in the cartilage, and in the mucous glands where under the present conditions of histological treatment, it has been retained as synthesized chondroitin (Dziewiatkowski, '51b) or mucoitin sulfate. The mucous elements have also exhibited apparent differences in regards to sulfur synthesis. The sero-mucous olfactory glands appeared to be less active in the incorporation of sulfur than do the mucous salivary glands which have also a more rapid turnover.

On the other hand, the stomach has shown very apparent differences between the three classical regions of the glandular portion and also between the neck cells and the surface cells. These pictures reinforce the theory of duality between surface mucous cells and mucous neck cells arrived at by tinctorial variations (Sóeborg-Ohlsen, '41; Leblond, '50; Stevens and Leblond, '53). They also indicate strongly that the mucous neck cells as well as the mucous cells of the cardiac and pyloric glands are responsible for the secretion of the sulfomucoprotein present in the gastric secretion (Levene and Lopès-Suarez, '16; Webster and Komarov, '32; Glass and Boyd, '49; Bélanger, '53b).

A clear cut difference in regards to sulfur incorporation has also been established by the present experiments between the pyloric gland and the duodenal gland of Brünner which under comparable conditions has remained constantly negative (figs. 3 and 4) in the autoradiographs.

In tissues of mesodermic origin, apart from cartilage, the radiosulfate has been localized in mineralizing bone (Dziewiatkowski, '51a, Bélanger, '53) and dentine (Bélanger, '53) where it seems to precede mineral deposition and then become part of an organic-mineral complex which remains bound once formed. It has been reported here to enter in the composition of tendons, ligaments, aponeuroses, mesenteries, capsules and trabeculae. It has been seen in basement membranes, in the lung, in the wall of the heart, blood vessels, and glandular ducts, in increasing proportions as the structures become larger. It has been seen in the sclera, the cornea, the dental pulp (Bélanger, '53), the papilla and capsule of the hair follicles and also in the mast cells (fig. 10). In this particular location, the fixed S^{35} can be ascribed to the mucoitin polysulfuric acid heparin (Holmgren and Wilander, '37) or to a precursor form (Asbol-Hansen, '53), confirming the theory of production of this substance by these cells. The other localizations bear a definite relationship to collagen and reticulin. Since the radioactive areas recognized so far have been ascribed to specific muco- or glucoproteins with a sulfopolysaccharide fraction, it seems logical to consider these more generalized locations as belonging to a large distribution of such substances existing in close relationship with the filamentous collagen and reticulin (interfibrillar cement). This opinion has been expressed previously by Gersh and Catchpole ('49), Layton ('51) and Boström and Odeblad ('53). On the other hand, Böstrom and Gordell ('53) have demonstrated that sulfate is utilized in a very small extent in the synthesis of sulfur-containing amino acids, taurine, cystine and methionine.

The fact that toluidine blue metachromasia is indicative of the presence of sulfopolysaccharides (Lison, '53; Pearse, '53)

is strongly reinforced by the present concurrent evidence of sulfate autoradiographs. In appreciating metachromasia, we have followed the advice of Pearse ('53) who states that "in a true histochemical test, no differentiation must be employed."

The rapid disappearance of radiosulfur over all areas of connective tissue within two days with the exception of the mast cells, seems to indicate that these cells are storing as well as producing.

The localizations to the enamel of the growing teeth, and to the membranes of the inner ear, have been reported and discussed elsewhere (Bélanger, '54).

SUMMARY

Following a subcutaneous injection of S^{35} labeled H_2SO_4, the radiosulfur has been localized by autoradiography to various tissues and organs, with a large range of relative radioactivity.

The mineralizing tissues and the mucous glands have exhibited the largest synthesis, with the exception of the surface cells of the stomach and the Brünner glands of the duodenum which do not seem to pick up radiosulfur.

The mast cells of connective tissue and all areas rich in collagen have taken in radiosulfur.

The region of the basement membrane in the gastro-intestinal tract and genito-urinary system have also shown evidence of sulfate fixation.

The medulla of the kidney and the megakaryocytes of the spleen have also given positive S^{35} autoradiographs.

All these localizations seem related to the building up and concentration of sulfopolysaccharides and stain metachromatically with toluidine blue.

The radiosulfur has disappeared within two days from all mucous glands except from the olfactory mucosa. The connective tissue seems to have lost most of its organic bound sulfate at the end of that period with the exception of the mast cells which were still strongly positive after 4 days.

ACKNOWLEDGMENTS

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LITERATURE CITED

ASBOL-HANSEN, G. 1953 Autoradiography of mast cells in experimental skin tumours of mice injected with radioactive sulfur (S^{35}). Cancer Res., *13:* 587–589.

BÉLANGER, L. F. 1950 A method for routine detection of radiophosphates and other radioactive compounds in tissues: the inverted autograph. Anat. Rec., *107:* 149–160.

———— 1952 Improvements to the melted emulsion technique of autoradiography. Nature, *170:* 625.

———— 1952 Autoradiographic and histochemical observations on the mineralization of teeth in rats and hamsters of various ages. Anat. Rec., *114:* 529–554.

———— 1953 Autoradiographic and histochemical studies of the entry and incorporation of sulphur in growing bones and teeth of the rat. Proc. 19th Int. Physiol. Cong., 199–200.

———— 1953 Autoradiographic detection of S^{35} in the membranes of the inner ear of rat. Science, *114:* 520–521.

———— 1953 Autoradiographic detection of S^{35} synthesis by the mucous neck cells of the stomach of rat. Nature, *172:* 1150.

———— 1954 Autoradiographic visualization of the entry and transit of S^{35} in cartilage, bone and dentine of young rats and the effect of hyaluronidase in vitro. Can. J. Biochem. and Physiol., May.

BÉLANGER, L. F., AND C. P. LEBLOND 1946 A method for locating radioactive elements in tissues, by covering histological sections with a photographic emulsion. Endocrinology, *39:* 8–13.

———— 1950 Mineralization of the growing tooth as shown by radiophosphorus autographs. Proc. Soc. Exp. Biol. and Med., *73:* 390–391.

BOSTRÖM, H., AND S. GORDELL 1953 Uptake of sulphates in mucopolysaccharides esterified with sulphuric acid in the skin of adult rats after intraperitoneal injection of S^{35} labelled sodium sulfate. Acta Chem. Scand., *7:* 216–222.

BOSTRÖM, H., AND E. ODEBLAD 1953 Autoradiographic observations on the incorporation of S^{35} labeled sodium sulphate in the rabbit foetus. Anat. Rec., *115:* 505–514.

DZIEWIATKOWSKI, D. D. 1949 Rate of excretion of radioactive sulphur and its concentration in some tissues of the rat after intraperitoneal administration of labeled sodium sulfate. J. Biol. Chem., *178:* 197–202.

———— 1951a Radioautographic visualization of Sulfur 35 disposition in the articular cartilage and bone of suckling rats following injection of labeled sodium sulfate. J. Exp. Med., *93:* 451–458.

———— 1951b Isolation of chondroitin sulfate S^{35} from articular cartilage of rats. J. Biol. Chem., *189:* 187–190.

GERSH, I., AND H. R. CATCHPOLE 1949 Ground substance and basement membrane. Am. J. Anat., *85:* 457–522.

GLASS, G. B. J., AND L. J. BOYD 1949 The three main components of the human gastric mucin: dissolved mucoprotuse, dissolved mucoprotein and mucoid of the gastric visible mucus. Gastroenterology, *12:* 821–878.

HOLMGREN, H., AND O. WILANDER 1937 Beitrag zur kenntnis der chemie und funktion der ehrlichschen mastzellen. Ztschr. f. mikr. Anat.-Forsch., *42:* 242–278.

LAYTON, L. L. 1951 The anabolic metabolism of radioactive sulfate by animal tissues in vitro and in vivo. Cancer, *4:* 198–201.

LEBLOND, C. P. 1950 Distribution of acid reactive carbohydrates in the adult rat. Am. J. Anat., *86:* 1–49.

LEBLOND, C. P., G. W. WILKINSON, L. F. BÉLANGER AND J. ROBICHON 1950 Visualization of bone formation in the rat with the help of P^{32} radioautographs. Am. J. Anat., *86:* 289–341.

LEVENE, P. A., AND J. LOPEZ-SUAREZ 1916 Conjugated sulfuric acid of the mucin of pig's stomach (Mucoitin sulfuric acid). J. Biol. Chem., *25:* 511–516.

LISON, L. 1953 Histochimie et cytochimie animales (2nd Ed.). Gauthier-Villars, Paris.

MELARAGNO, H. P., AND W. MONTAGNA 1953 The tactile hair follicles in the mouse. Anat. Rec., *115:* 129–150.

PEARSE, A. G. E. 1953 Histochemistry theoretical and applied. J. and A. Churchill Ltd., London.

SØEBORG-OHLSEN, A. 1941 Contribution to the histochemistry of the stomach. C. R. travaux du lab. de Carlsberg (série chimique), *23:* 329–478.

STEVENS, C. E., AND C. P. LEBLOND 1953 Renewal of the mucous cells in the gastric mucosa of the rat. Anat. Rec., *115:* 231–246.

SYLVEN, B. 1950 The qualitative distribution of the metachromatic polysaccharide material during hair growth. Exp. Cell Research, *1:* 582–589.

WEBSTER, D. R., AND S. A. KOMAROV 1932 Mucoprotein as a normal constituent of the gastric juice. J. Biol. Chem., *96:* 133–142.

PLATE 1

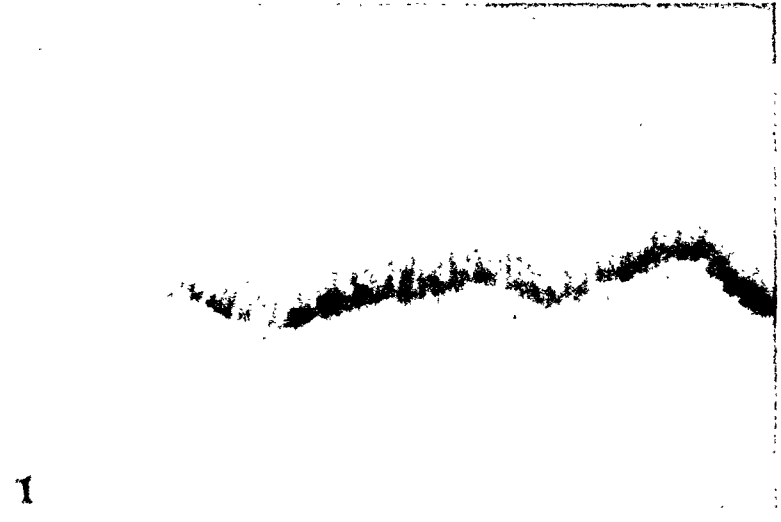
EXPLANATION OF FIGURES

1 Proximal portion of the glandular stomach in a 10 day old rat sacrificed one hour after a subcutaneous injection of S^{35}. Inverted autoradiograph, × 32. The glands in this area have incorporated S^{35} along most of their length.

2 Middle portion of the stomach from the same preparation. The portion of the glands which incorporate S^{35} has decreased in proportion and on the right hand side, it is seen to occupy only a small band, the neck of the gland.

3 Pyloric region of a 4 day old rat sacrificed one hour after a subcutaneous injection of S^{35}. Inverted autoradiograph, × 32. The pyloric glands have incorporated the radiosulfur and are seen to end abruptly on the duodenal side of the pylorus. The apparent absence of S^{35} on the upper side of the picture is due to the incidence of section. The Brünner glands are negative.

4 Pyloric region of a 10 day old rat sacrificed two hours after a subcutaneous injection of S^{35}. Inverted autoradiograph, × 32. There is a strong reaction all over the pyloric gland. The Brünner glands are negative. Individual pinpoint reactions over the duodenal villi indicate the position of the mucous goblet cells.



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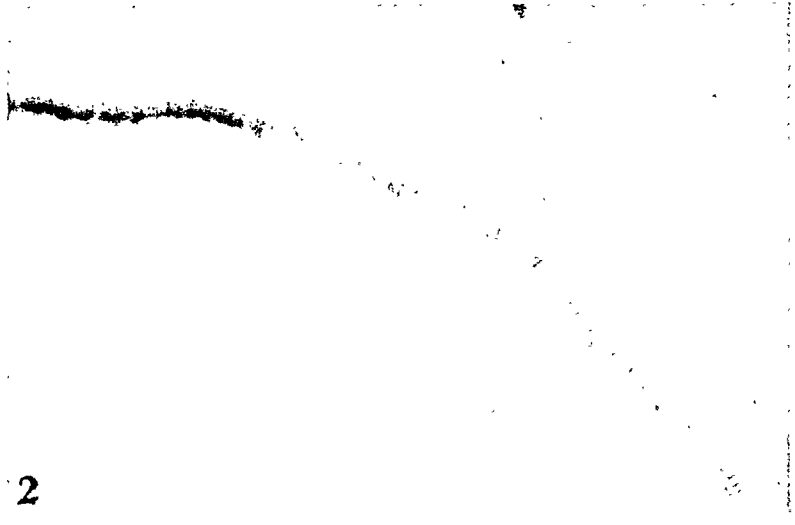

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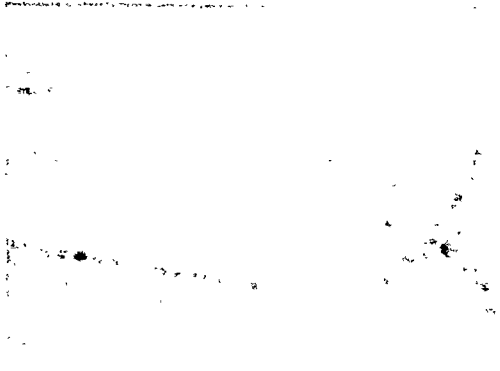


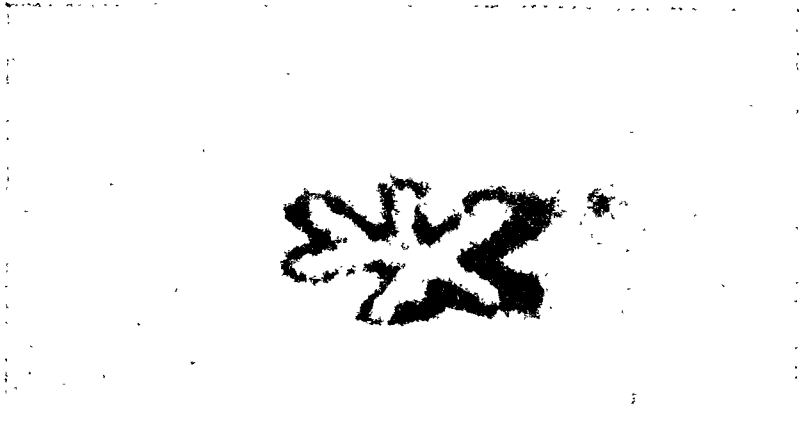


PLATE 2

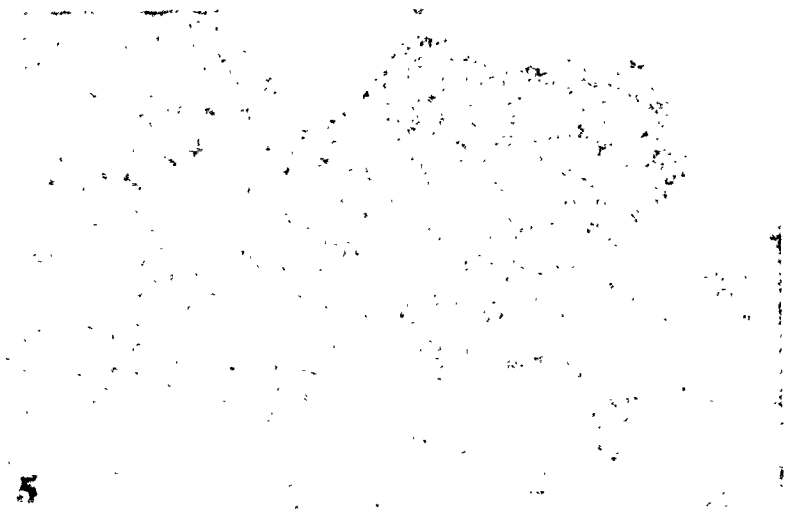
EXPLANATION OF FIGURES

5 Intestinal villi from the same animal as in figure 4. The individual goblet cells show an autoradiographic picture. × 108.

6 Section of the colon from the same animal as in figure 4. There is a strong reaction over the mucus cells of the mucosa and also some radioactive free mucus in the lumen. × 32.

7 The sub-lingual and sub-maxillary glands of a 4 day old Hamster sacrificed two hours after a subcutaneous injection of S^{35}. Inverted autoradiograph of celloidin section, × 52. The large sub-lingual mucous gland shows a strong overall reaction, while the smaller sub-maxillary gland on the right shows only a few pin-point reactions over the mucous cells of the mixed acini.

8 Middle portion of the glandular stomach in a rat 12 days of age, injected two days previously with S^{35}. Inverted autoradiograph, × 52. There is no reaction over the mucosa but radioactive streaks at the periphery of the stomach content indicate the presence of non-soluble secretion from the glands.



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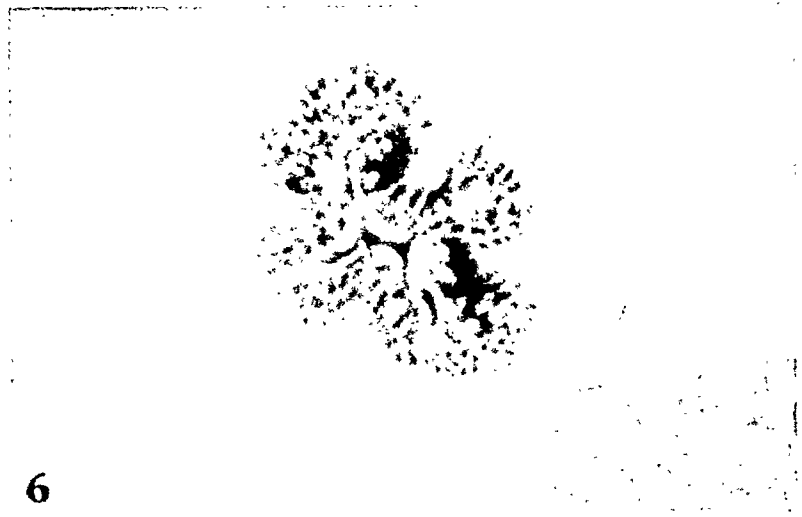

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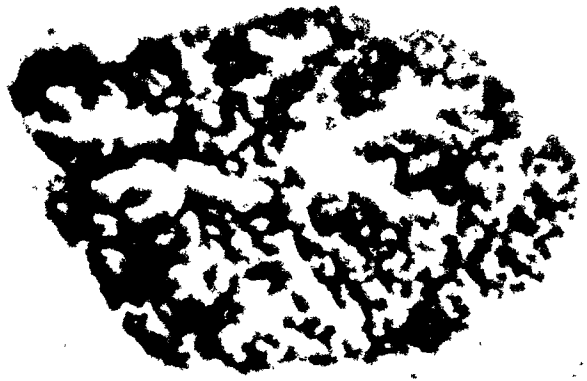


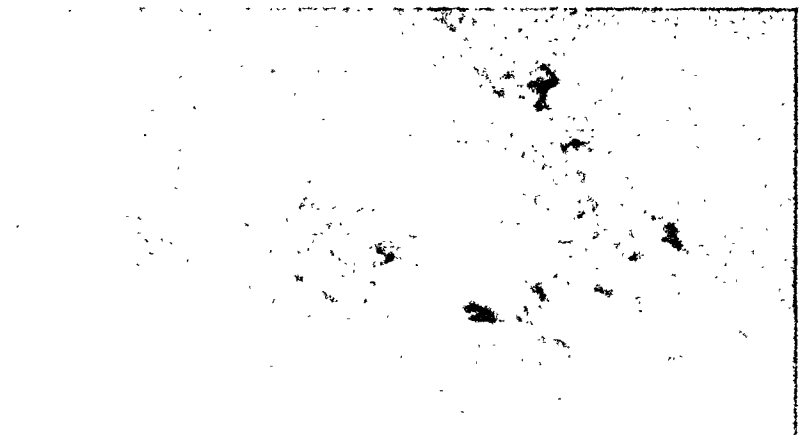


PLATE 3

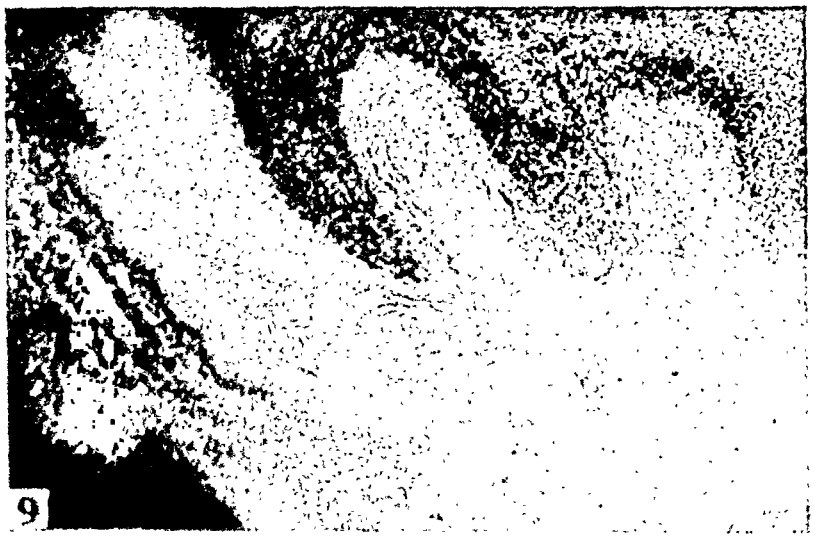
EXPLANATION OF FIGURES

9 The non-glandular portion of the stomach of a 10 day old rat, sacrificed two hours after a subcutaneous injection of S^{35}. Inverted autoradiograph, × 116. Exposure 90 days. There is a large uniform band of reaction immediately behind the squamous stratified epithelium. A small portion of glandular epithelium is responsible for the strong autoradiograph visible in the lower left corner.

10 A portion of subcutaneous tissue from the lateral region of the face of a 4 day old rat sacrificed two hours after a subcutaneous injection of S^{35}. Inverted autoradiograph, celloidin section, × 32. The mast cells show strong punctiform reactions; the outer wall of the follicles and also the papillae of the tactile hairs show a moderate reaction after an exposure of 90 days.

11 The kidney of a 4 day old rat sacrificed two hours after a subcutaneous injection of S^{35}. Inverted autoradiograph, × 32. Exposure 90 days. The pyramid and the medullary rays show a moderate reaction. The capsule area is also mildly positive.

12 The pelvic area in the same animal as in figure 11. Inverted autoradiograph, × 32. Exposure 90 days. The wall of the blood vessels, particularly that of the larger arteries, show an intense reaction. The border of the renal pelvis (lower left hand corner of figure and also fig. 11) as well as the area of tissue immediately behind the epithelium of the ureter show a reaction. There are a few punctiform autoradiographs from the mast cells.



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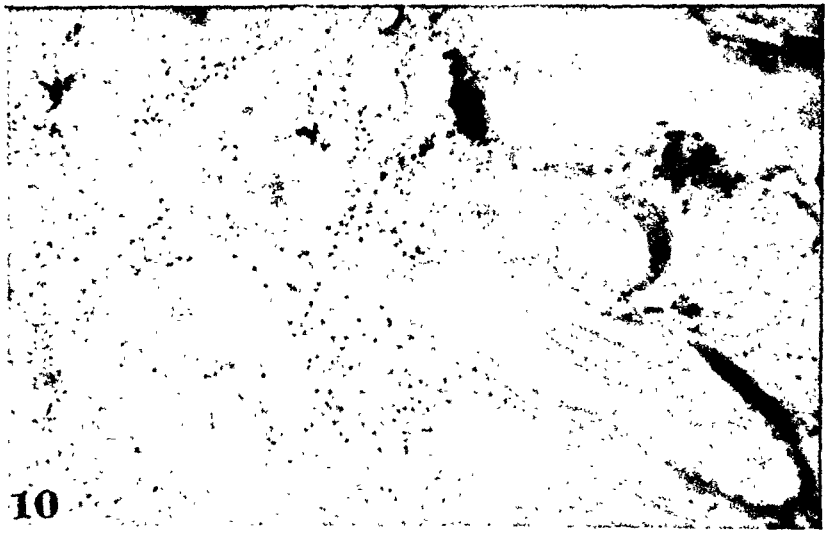

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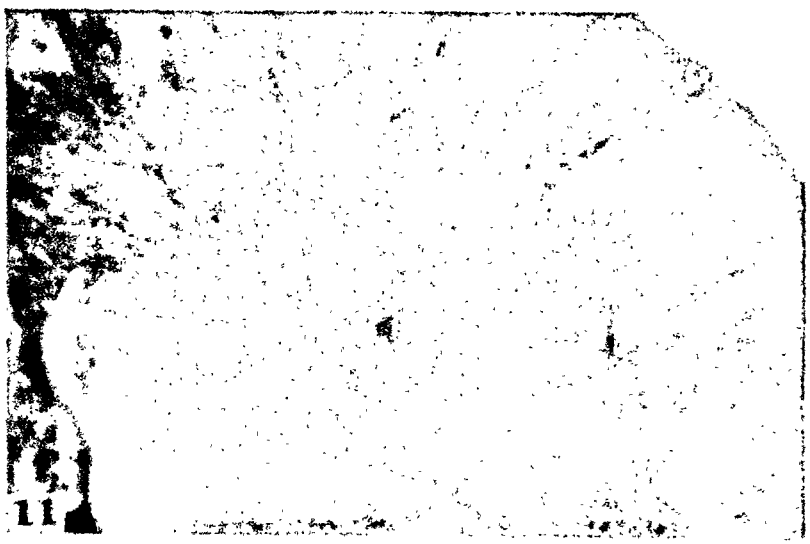

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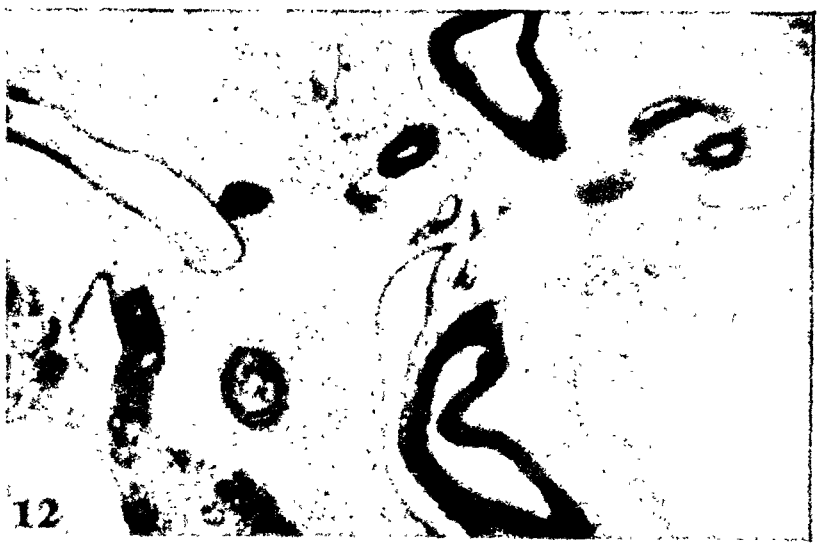

12

MORPHOGENESIS AND METABOLISM OF AMPHIBIAN LARVAE AFTER EXCISION OF HEART

II. MORPHOGENESIS OF HEARTLESS LARVAE OF AMBLYSTOMA PUNCTATUM [1]

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ELEVEN FIGURES

INTRODUCTION

In the previous paper of this series (Kemp, '53) it was reported that tadpoles of Rana pipiens would live up to 6 days at room temperature after excision of heart at Shumway stage 22 or 23. Because of the obvious differences between anuran and urodele larvae in form and rate of development, the experiment was repeated on Amblystoma punctatum at Harrison stage 40, 41 or 42 (Kemp and Quinn, '51). It was interesting to find that heartless larvae of Amblystoma survived at room temperature up to 15 days — more than twice as long as tadpoles of Rana. Although there are many similarities in differentiation of circulationless larvae of Rana and Amblystoma, e.g., microcephaly, collapse of the vitreous chamber of the eye, swelling of pronephric tubules, postcardinal sinuses and dorsal mesentery, reduced motility and slow utilization of yolk, there are also important differences. Lymph spaces in the body wall, in which fluid may accumulate, are pronounced in Rana but negligible in Amblystoma. Anterior limb buds and balancers, on the other hand, are present in Amblystoma but absent in developing tadpoles.

[1] Aided by a grant from the Horace H. Rackham School of Graduate Studies, University of Michigan.

Heartless larvae of Amblystoma, furthermore, exhibit albinism due to concentration of melanin within the melanophores.

MATERIALS AND METHODS

At Harrison stage 40, 41 or 42 the heart of Amblystoma is closer to the ventral surface than is the heart of Rana at morphologically equivalent stages; hence it is easier to remove. Larvae were anesthetized by electric shock and the pericardial cavity opened by making a midventral incision through the body wall with the Burch ('42) type of microscalpel. The heart was excised by cutting through the bulbus arteriosus and the sinoatrial region. A total of 240 animals were cultured after cardiectomy, either in plain Holtfreter's solution or in Holtfreter's solution containing 0.5% to 1.0% sodium sulfadiazine as recommended by Detwiler and Robinson ('45). Survival beyond three days after operation was unusual in simple salt solution but was so increased with sulfadiazine present that 80% of the larvae cultured in the antibiotic solution survived up to 7 days, and a few survived longer — to a maximum of 15 days for one specimen. One or two days after operation, animals were transferred to pond water. At daily intervals after operation, specimens were fixed in Bouin's fluid for subsequent sectioning. Slides were routinely stained with hematoxylin and eosin. Photographs of whole animals were made with a Kine-Exacta camera and of sections with a Spencer photomicrographic camera.

OBSERVATIONS

A. Gross changes

Experimental animals could be distinguished from controls as early as one day after excision of heart. By this time both the main stem of the gills and their secondary filaments had elongated farther in the controls. During subsequent days the gills in normal animals grew larger, while those in experimental animals gradually became shorter because of degeneration at the tips. Development of the limbs provided

another distinctive contrast between normal and experimental animals. Limbs of the latter remained relatively short and never became flexed at the elbow joint; nor did they ever become more than slightly bifurcated into two digits distally. In normal animals, on the other hand, the limb elongated, became flexed at the elbow, underwent pronation and developed three digits during the period studied. As in Rana (Kemp, '53), the eyes remained small and immovable in circulationless larvae. Also as in Rana they developed in association with microcephaly of the anterior part of the head. Most of the epidermal melanophores around the head were maximally contracted to a small dot in heartless animals as early as one day after cardiectomy, likewise most of the dermal melanophores along the ventral borders of the pigmented flanks. In subsequent days more and more of the dermal melanophores became contracted until eventually the animals became "albino" (fig. 1). Experimental animals first exhibited swelling in the region of the pronephroi and anterior digestive organs of the coelom about two days after operation. Neither the head nor the posterior part of the coelomic cavity became appreciably swollen. Through the transparent body wall it could be seen that differentiation of the digestive tract was proceeding but at a slower rate in heartless animals than in normal ones.

B. Microscopic changes

1. Eye. At the time of operation the eye was well along in differentiation. Figure 2 illustrates a typical specimen showing the ganglionic layer already separated from the outer cells of the retina, but there is no demarcation between inner and outer nuclear layers. Rod cells are growing out into the space between retina and tapetum. Sclerosis has begun in the lens. Just as in Rana (Kemp, '53), the eye of Amblystoma suffers collapse of the vitreous chamber within the first day after excision of heart. For this reason the eye (fig. 3) appears smaller than that of a normal animal (fig. 4).

One day after operation the rods in eyes of experimental animals were no better developed than at the time of operation, whereas in control specimens they were longer. Pycnosis of some nuclei in the central part of the retina and in the mantle layer of the infundibulum was observed in one specimen fixed only two days after operation. Pycnosis was also observed in some cells of the eye and brain of several older specimens, but even in the oldest animal sectioned (14-day) most of the cells in these locations looked normal.

2. Pronephros and gastroduodenal region. Distention of pronephric tubules and postcardinal sinuses was apparent by the first day after operation. Fluid had obviously begun to accumulate and cause swelling of the dorsal aorta, dorsal mesentery, and the coelom of the gastroduodenal region. Continued uptake of fluid in subsequent days resulted in the development of large spaces beneath the mesodermal sheath of esophagus, stomach and duodenum or even in complete separation of the mesodermal sheath from the endodermal lining of the gastroduodenal portion of the digestive tract (figs. 6, 7). Hemopoiesis apparently continued within the spaces of the distended mesenteries of some specimens, for the blood cells in these spaces in older specimens often contained yolk platelets, whereas the red blood cells circulating at the time of operation were already yolkless.

3. Intestine. Yolk still filled the cells of the primitive intestine (fig. 5) at the time of operation. As might be expected, differentiation and digestion of yolk proceeded faster in normal animals than in experimentals. Neither group showed much change during the first post-operative day, but separation of the intestinal epithelium from the central *Nahrdotter* was beginning in the controls on the second day. By three days the epithelium in controls was completely formed and yolk had entirely disappeared from the intestinal lumen, although it was still abundant within the epithelial cells. Epithelium was completely defined in experimental animals by 4 days, but partially digested yolk still filled the lumen. No significant advance had occurred by the 6th day (figs. 8,

9). About the 7th day in controls, intestinal epithelial cells began to show vacuolar spaces of various sizes, possibly derived from digested yolk platelets. Holtfreter ('46) has described similar vacuoles in the larval epidermis of Rana pipiens and Hibbard ('28) has reported them in larvae of Discoglossus. Intact yolk platelets had practically all disappeared in control animals of 8 days or older but were abundant even in the oldest (14-day) experimental animal examined.

4. Spleen. The primordium of the spleen was recognizable in the splanchnic mesoderm adjacent to the stomach even at the time of operation. In heartless animals there was no further differentiation of spleen. In control animals, on the other hand, the spleen was noticeably larger one day after the stage of operation. By three days it was well developed. These observations indicate that circulating blood is an important factor in normal morphogenesis of the spleen.

5. Limb. At the time of operation the limb consisted of a core of proliferating mesodermal cells surrounded by epidermis (fig. 5). Blood vessels were present and at the base of the limb was a central group of precartilage cells, the primordium of the humerus. Yolk platelets were still present though scarce. Two days later control specimens possessed primordia of radius and ulna in addition to the humerus, whereas radius and ulna could not be distinguished in experimental animals until the third day. By this time the controls were definitely further advanced with respect to differentiation of cartilage. By 7 days the limb in heartless animals was only slightly longer and more differentiated than at three days, while in controls the limb had become flexed at the elbow joint and possessed well developed cartilages, including those in two well formed digits and a beginning third digit. In subsequent days there was virtually no further advance in development of the limb in experimental animals.

6. Skull and vertebral cartilages. Retardation in the rate of differentiation of cartilage in the limb was paralleled in the cranial, visceral and vertebral cartilages. Anlagen of

these cartilages were sufficiently differentiated at the stage of operation that they could be easily identified. After cardiectomy, however, there was very little further differentiation of cartilage. There was increase in basophilia of the matrix in some animals; but active multiplication of cells and marked growth, which characterized the cartilages of controls, did not occur in the experimental group (figs. 10, 11).

DISCUSSION

As stated previously (Kemp, '53), we may consider anomalies in circulationless larvae as resulting chiefly from reduced metabolism, from altered hydrostatic pressure, or from a combination of these factors. In the present study Amblystoma larvae lived up to 15 days after excision of heart, compared to only 6 days for the Rana tadpoles studied in the previous investigation. Measurements of oxygen consumption in animals of both species (Kemp, unpublished) reveal that Amblystoma respires about half as fast as Rana at comparable stages after the start of circulation. It can be inferred that the longer survival of heartless Amblystoma larvae is correlated with their relatively slow metabolism. Morphological evidence for reduced metabolism in these larvae is afforded by the behavior of melanophores, the retarded rate of utilization of yolk in the digestive tract, and the inhibition of both differentiation and growth in the spleen, gills, limbs and skeletal cartilages.

It is well known that a hormone, intermedin, elaborated by the intermediate lobe of the pituitary gland is responsible for the spreading out of the pigment granules in the melanophores of Amphibia. A number of workers, including Smith ('16, '20), Allen ('17), Blount ('32, '45), Atwell ('21), Eakin ('39) and Burch ('46) have shown that larvae lacking the intermediate lobe develop with the "albino" syndrome, characterized by concentration of melanin in both epidermal and dermal melanophores and maximal dispersion of pigment in the xantholeucophores. Of particular interest in the present investigation was the variability of response of individual

cells. Apparently the epidermal melanophores as a group are more sensitive to lack of a circulating factor, presumably intermedin, than are the dermal melanophores, since practically all of the former exhibited concentration of melanosomes within the first day after cessation of circulation. The response of the dermal melanophores, on the other hand, was variable. Those farthest ventral along the flanks tended to contract first, but as time went on the response spread dorsad so that eventually practically all of the dermal melanophores were contracted. Because of the variability in the time of response of these cells, it seems reasonable to hypothesize that the intermedin available became depleted at different rates. It is likely that a threshold level of the hormone must be maintained in the vicinity of the melanophores in order that maximal expansion of the pigment may occur. If this be true, the melanophores might remain in the expanded state only as long as the local supply of intermedin lasted or until it fell below the threshold level, or as long as the intermedin-induced reaction persisted after intermedin itself fell below the critical level.

Slow utilization of yolk in the digestive tract was anticipated in the present investigation because of the results of previous studies (Kemp, '51, '53) on anuran larvae. Blood circulating to the gut supplies something which increases the rate of digestion of yolk. Whether the circulating factor is merely oxygen or some other substance which might stimulate metabolism is unknown. It would indeed be surprising if only one substance were involved. The finding that circulating blood is important for development of the spleen is not surprising in view of the close association of the spleen with the circulatory system. Regression of gills and failure of the limbs to differentiate much beyond their condition at the time of excision of heart afford evidence that circulation is important for normal differentiation of these structures. Wilde ('52) has shown that presumptive gills have a high potential for growth and differentiation in tissue culture, even to the extent of suppressing differentiation of adjacent

limb primordia. The present study demonstrates, however, that continued growth and development of gills already well differentiated is dependent on circulation. This conclusion is in agreement with Moser's ('40) statement that "doubtless the lack of vascular connections partially, at least, explains the failure of continued differentiation of the gills." Wilde ('50) has also demonstrated that limb buds from embryos at stages 40 and 41 could differentiate humerus, radius and ulna, carpal mass and two digits in vitro. The primordium of a third digit developed in an explant from stage 43. Wilde's experiments showed that considerable organotypic growth and differentiation may take place in vitro, but it is significant that isochronous controls always showed much better development of the limb cartilages. Circulation of blood in the controls is the obvious key to this difference. The present study has shown that not only the limb cartilages but also all other cartilages of the larva are dependent on circulation for normal morphogenesis. Both Wilde ('50) and Fell and Robison ('29) have demonstrated that the degree of organotypic development in vitro depends on the stage of isolation of an explanted rudiment. One would expect that as an embryo ages it produces and stores more and more of the material needed for the synthetic activities of morphogenesis. Having more already in the warehouse would permit an older rudiment to proceed farther when isolated. Possible explanations for the poor development of cartilage in heartless animals of the present investigation are (1) that the primordia for this tissue had not accumulated sufficient reserves to permit long-continued differentiation and growth after cessation of circulation or (2) that the rate of metabolism in these primordia fell too low. One particular metabolite which appears to be implicated in the differentiation of both young cartilage and bone is alkaline phosphatase (Karczmar and Berg, '51).

Accumulation of fluid in body spaces — pronephric tubules, postcardinal sinuses, dorsal aorta, dorsal mesentery and beneath the mesodermal sheath of the gut — occurs in Amblystoma much as previously described for Rana (Kemp, '53).

One obvious difference between the two species is that in Rana fluid collects in lymph spaces of the body wall, causing the anterior end to broaden markedly. This does not occur in Amblystoma. Collapse of the vitreous chamber of the eye evidently results from the inability of the eye to maintain normal intraocular hydrostatic pressure in animals lacking circulation. Bodenstein ('48) and Bodenstein and Goldin ('48) have published photographs showing collapsed vitreous chambers in the eyes of Amblystoma larvae subjected to nitrogen mustard. It has been suggested (Kemp, op. cit.) that these and many other abnormalities induced by exposure of embryos to various drugs or radiations may well be secondary effects resulting from impaired circulation.

SUMMARY

1. Larvae of Amblystoma punctatum rendered circulationless by excision of heart at Harrison stage 40, 41 or 42 survived at room temperature up to 15 days.

2. Gross examination revealed a gradual regression of gills and only slight further development of limbs after cardiectomy. Eyes remained small and immovable and the head became microcephalic. Melanophores became contracted so that heartless animals were "albino." Internal swelling was pronounced in the gastroduodenal region. Differentiation of digestive tract continued but was considerably slower than normal.

3. Microscopic examinations revealed that the vitreous chamber of the eye became collapsed in circulationless animals. Pronephric tubules, postcardinal sinuses, dorsal aorta, dorsal mesentery, spaces beneath the mesodermal sheath of the gut, and the coelom in the gastroduodenal region became swollen. Utilization of yolk was conspicuously retarded in the intestine and the spleen stopped developing after circulation ceased. Cartilage in the limbs, skull and vertebral primordia differentiated only slightly after the heart was removed.

4. Circulating blood is important for regulating hydrostatic pressure in Amblystoma larvae, as well as for supplying oxy-

gen and other materials needed for the continual synthetic activity of differentiation and growth.

LITERATURE CITED

ALLEN, B. M. 1917 Effects of the extirpation of the anterior lobe of the hypophysis of Rana pipiens. Biol. Bull., *32:* 117–130.

ATWELL, W. J. 1921 Further observations on the pigment changes following removal of the epithelial hypophysis and the pineal gland in the frog tadpole. Endocrinology, *5:* 221–232.

BLOUNT, R. F. 1932 Transplantation and extirpation of the pituitary rudiment and the effect upon pigmentation in the urodele embryo. J. Exp. Zool., *63:* 113–141.

———— 1945 The interrelationship of the parts of the hypophysis in development. J. Exp. Zool., *100:* 79–101.

BODENSTEIN, D. 1948 The effects of nitrogen mustard on embryonic development. II. Effects on eye development. J. Exp. Zool., *108:* 93–125.

BODENSTEIN, D., AND A. GOLDIN 1948 A comparison of the effects of various nitrogen mustard compounds on embryonic cells. J. Exp. Zool., *108:* 75–91.

BURCH, A. B. 1942 A microscalpel for use in experimental embryology. Science, *96:* 387–388.

———— 1946 An experimental study of the histological and functional differentiation of the epithelial hypophysis in Hyla regilla. Univ. Calif. Publ. Zool., *51:* 185–213.

DETWILER, S. R., AND C. O. ROBINSON 1945 On the use of sodium sulfadiazine in surgery on amphibian embryos. Proc. Soc. Exp. Biol. and Med., *59:* 202–206.

EAKIN, R. M. 1939 Correlative differentiation of the intermediate lobe of the pituitary in Triturus torosus. Growth, *3:* 373–380.

FELL, H. B., AND R. ROBISON 1929 The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated in vitro. Biochem. J., *23:* 767–784.

HIBBARD, H. 1928 Contribution à l'étude de l'ovogenèse, de la fécondation, et de l'histogenèse chez Discoglossus pictus Otth. Arch. de Biol., *38:* 251–326.

HOLTFRETER, J. 1946 Experiments on the formed inclusions of the amphibian egg. III. Observations on microsomes, vacuoles, and on the process of yolk resorption. J. Exp. Zool., *103:* 81–112.

KARCZMAR, A. G., AND G. G. BERG 1951 Alkaline phosphatase during limb development and regeneration of Amblystoma opacum and Amblystoma punctatum. J. Exp. Zool., *117:* 139–163.

KEMP, N. E. 1951 Development of intestinal coiling in anuran larvae. J. Exp. Zool., *116:* 259–287.

———— 1953 Morphogenesis and metabolism of amphibian larvae after excision of heart. I. Morphogenesis of heartless tadpoles of Rana pipiens. Anat. Rec., *117:* 405–425.

KEMP, N. E., AND B. L. QUINN 1951 Differentiation of Amblystoma larvae after extirpation of heart. (Abstract.) Anat. Rec., *111:* 543–544.

MOSER, F. 1940 The differentiation of isolated gills of the amphibian embryo. J. Morph., *66:* 261–276.

SMITH, P. E. 1916 The effect of hypophysectomy in the early embryo upon the growth and development of the frog. Anat. Rec., *11:* 57–64.

———— 1920 The pigmentary, growth and endocrine disturbances induced in the anuran tadpole by the early ablation of the pars buccalis of the hypophysis. Am. Anat. Mem., *11:* 1–51.

WILDE, C. E., JR. 1950 Studies on the organogenesis in vitro of the urodele limb bud. J. Morph., *86:* 73–114.

———— 1952 Studies on the organogenesis in vitro of the urodele limb bud. II. The effect of size of the explant and the interrelation between gill and limb. J. Morph., *90:* 119–148.

PLATE 1

EXPLANATION OF FIGURES

1 Dorsal views of control (left) and experimental larvae one day after operation. Contraction of most of the melanophores of the heartless larva is apparent. Those farthest ventral along the flank are contracted most. Eye of experimental larva is smaller than that of control. × 8.

2 Eye at stage of operation. Central part of retina is separated into ganglionic layer and nuclear layer. Rods are growing out into space between retina and tapetum. Sclerosis of fibers evident in center of lens. × 70.

3 Eye of heartless larva one day after operation. Collapse of vitreous chamber has brought lens and retina into direct contact. × 70.

4 Eye of control larva one day after stage of operation. Rods are slightly longer and more numerous than on previous day. × 70.

5 Intestine and limb of larva at stage of operation. Yolk platelets are closely packed throughout the intestine. Limb shows central blood vessel within mesodermal core. × 70.

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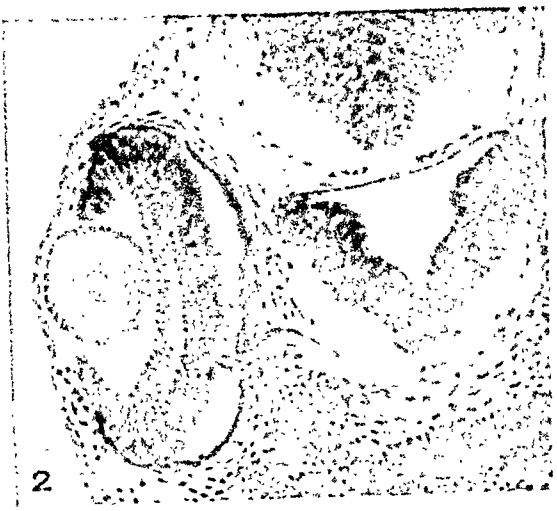

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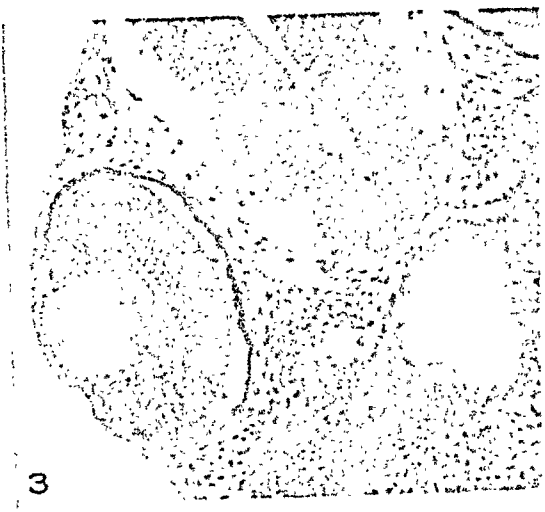

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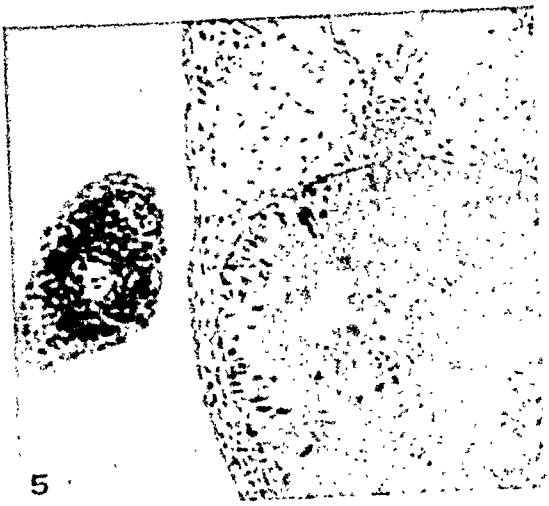

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PLATE 2

EXPLANATION OF FIGURES

6 Six-day experimental larva showing distention of pronephric tubules (pr), postcardinal sinus (ps), dorsal aorta (a), dorsal mesentery (m), space beneath mesodermal sheath of stomach (s), and coelom (c). × 70.

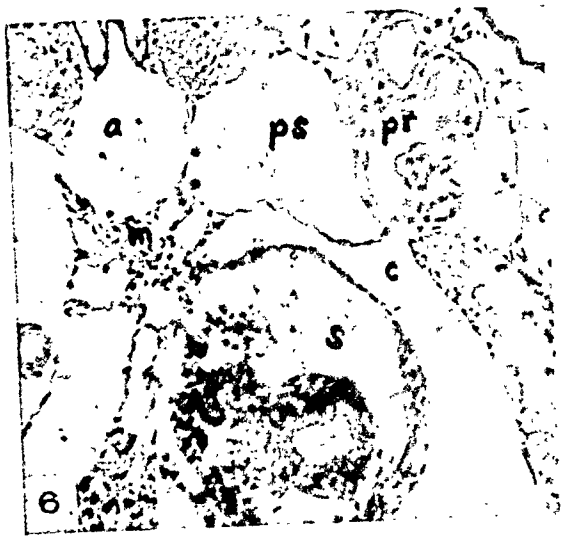
7 Six-day control larva at level of pronephric tubules (pr), stomach (st) and lung buds (l). × 70.

8 Intestine of 6-day experimental larva. Note ballooning of dorsal mesentery. Partially digested yolk occludes lumen and intestinal epithelium is packed with yolk platelets. × 70.

9 Intestine of 6-day control larva. Yolk digested from lumen but platelets still abundant in epithelium. × 70.

10 Section through auditory vesicle of 14-day heartless larva. Parachordal cartilages (pc), palatoquadrate cartilage (pq), and cartilage of auditory capsule (a) can be recognized but are poorly developed. × 70.

11 Fourteen-day control larva. Basilar plate of trabecular cartilages (b), palatoquadrate (pq), and auditory capsule (a) are well developed. × 70.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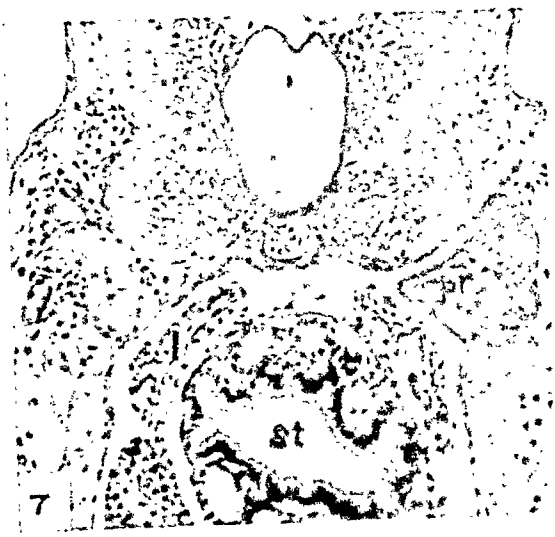
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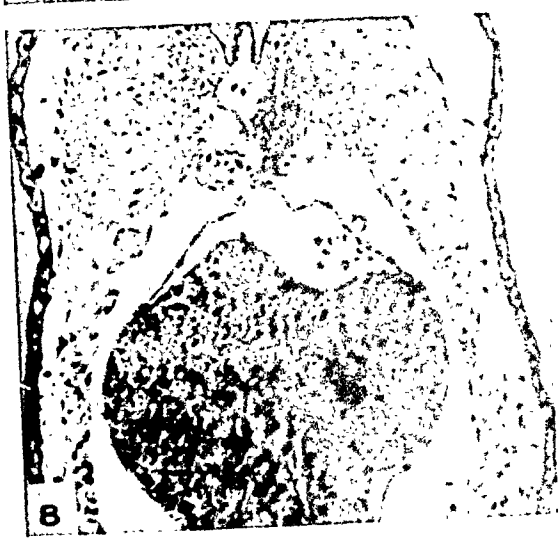
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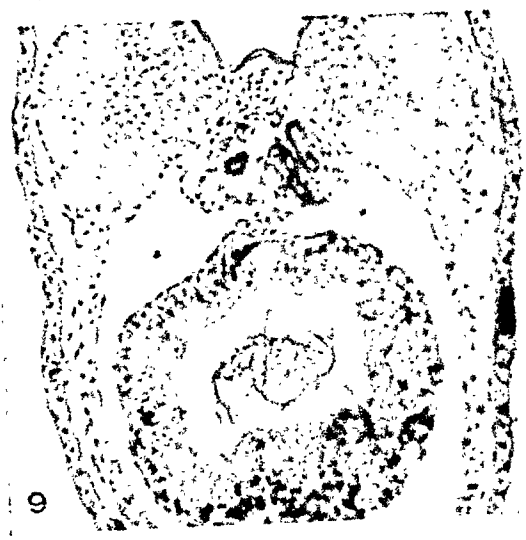
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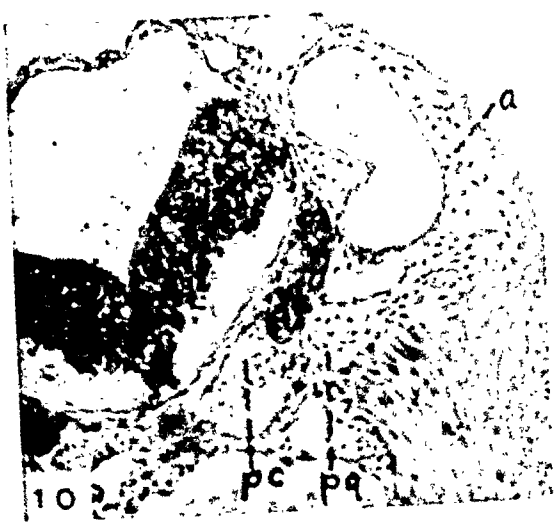
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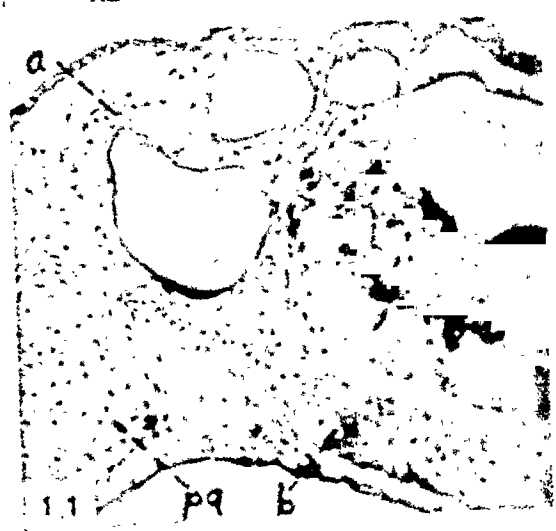
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NERVE TERMINATIONS IN THE MYOMETRIUM OF THE RABBIT

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TWELVE FIGURES

INTRODUCTION

General

In view of the physiological importance of the muscular tissue of the uterus, the vagueness and imperfection of our knowledge concerning the intrinsic innervation of the myometrium is deplorable. Review of the fairly extensive literature that has accumulated since Kilian (1851) first clearly stated the presence of nerve fibers in the uterine wall yields conviction only on the general point, that there are nerves in the myometrium. The exact relation of their terminal branches to the muscular tissue has been variously described. Some writers speak of a close connection between nerves and muscle, with nerve endings directly upon or even inside the muscular elements. Others have declared their inability to observe anything more intimate than a final ramification among the muscle bundles. Choosing from this wide range those descriptions which seem most plausible in the light of current knowledge of the nervous system, one gets the impression that there is a fairly rich terminal arborization, ending mostly in fine pointed twigs between the muscle cells, but sometimes provided with small terminal knobs which possibly

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lie upon the muscle cells. Among these accounts, which will be briefly reviewed below, not one is accompanied by illustrations which would satisfy a critical observer familiar with nerve endings in other parts of the body.

Recent advances in our knowledge of the physiology of uterine muscle now make necessary more exact knowledge of its innervation, in order that we may understand the functional motility of the uterus as a whole, in the complex mechanisms of gestation and parturition. Two aspects of uterine motility are involved. In the first place, the myometrial fibers, like other smooth muscle cells, have the property of spontaneous rhythmic contraction, as may be observed in excised strips of uterine muscle studied in vitro. Uterine muscle therefore is not dependent for its power to contract under physiological conditions, as is skeletal muscle, upon direct stimulation via the nervous system. The muscular tissue of the uterus in situ, however, responds to stimulation of the hypogastric nerves, as was clearly shown by Langley and Anderson (1895) in experiments on cats and rabbits. In the second place, its automatic contractility, and its response to oxytocic drugs are under control, as to rate, force and amplitude, by the ovarian hormones (see Reynolds, '49; and Corner and Csapo, '53, for discussion and literature). This endocrine control is obviously essential for normal functioning of the uterus in reproduction. On the other hand, the part played by nervous control in integrating the total activity pattern of the uterus is not clear. The denervated pregnant uterus, even the uterus isolated from the body, delivers itself of the fetuses by coordinated movements resembling those of the intact organ (see Fleming, '28, for references). The famous observations of Goltz and Freusberg (1874) showed that conception, implantation, gestation and spontaneous parturition can occur in a bitch after section of the spinal cord at the first lumbar segment. It is reported that rats (Bacq, '32) and cats (Cannon et al., '29) subjected to removal of the sympathetic ganglionated trunks are able to become pregnant and to bear young.

In spite of all this it is difficult to suppose that hormonal control, presumably generalized over the whole myometrium, can suffice for such elaborate functional integration, for example, as that which provides for the transport of early embryos to the site of implantation and their implantation at regularly spaced intervals along the uterine horns, as seen in animals with multiple litters such as the rabbit and dog. Some sort of peristaltic action must be involved such as that which is thought, in the case of the intestinal tube, to be regulated by the nerve plexuses of the muscular layers.

The answer to this more complex aspect of the problem (i.e., the nature of the coordination of the various segments of the uterus) depends in part on knowledge of the relation between muscle and nerve in the myometrium. In the present paper we shall undertake to discuss only the anatomical arrangement of the intrinsic uterine nerves.

Nervous pathway to the myometrium

Langley and Anderson (1895, 1896) showed by anatomical and physiological methods applied to the cat and rabbit, that the uterus receives its motor fibers from the sympathetic chain, chiefly from about the 4th to the 6th lumbar ganglia, via the presacral (hypogastric) nerve strands. In the rabbit as in the human species there are small ganglia in the parametrial plexus at the level of the cervix uteri, and the impulses to the uterine horns (or corpus uteri in the single-chambered species) are distributed by way of these ganglia and thence through the parametrium. The pattern of distribution beyond the ganglia has not been traced by anatomical methods in small animals such as the rabbit and cat, but Reynolds and Kaminester ('35) found by carefully devised stimulation experiments on rabbits that the nerves passing to the uterine musculature innervate successive, rather restricted regions and do not contribute to a widespread diffuse plexus within the uterine horns. Labhardt ('06) gives a diagram presumably based upon his own methylene blue and

Golgi preparations, which indicates that the nerve trunks entering the uterine horn from the parametrium pass into the connective-tissue layer between the outer longitudinal and inner circular coats of the myometrium and ramify longitudinally there, sending branches outward into the longitudinal layer and inward into the circular layer. Although these observations, taken together, fall far short of the detail necessary for complete understanding, they doubtless indicate the general topographic pattern of the parametrial and the major intra-uterine trunks.

Intrinsic innervation

Turning now to the problem of our own inquiries, we need quote only those who have investigated the subject in the past half-century. A few earlier writers who deserve credit for observations which now appear to have been more or less accurate (e.g., Patenko, Köstlin, Clivio, Gavronsky) will be found to have been cited by later writers quoted here.

It must be emphasized that everyone who has attempted to demonstrate the finer nerve endings in the myometrium has found this tissue especially difficult in comparison even with other viscera. Such bafflement is either explicitly stated by the investigators or is revealed by the sparsity of the terminal ramifications pictured by some of them. The explanation lies partly at least in a peculiar resistance of the intercellular spaces of the endometrium to the diffusion of fluids used in the staining processes. We shall refer to this difficulty again with a tentative explanation when describing our own experience. Thus neither the methylene blue technique nor impregnation with silver or gold as in the Bielschowsky, Cajal and Golgi methods succeed readily in revealing the finer branches. This major obstacle, needless to say, is augmented by the usual difficulties met with in observing fine details in a closely packed tissue. The metallic impregnations often obscure and modify the very structures which are under observation, or non-selectively obtrude ir-

relevant elements (connective tissue, precipitation masses, etc.) into the picture. Sectioning tends to break up the finest structures, and is, moreover, not readily applicable to the methylene blue preparations because of their chemical instability.

Labhardt ('06), who studied rabbit and human uteri in both Golgi and methylene blue preparations, reported that the nerve fibers end in fine twigs (Spitzen) within the fiber-bundles. He could not discover a close relation between the muscle cells and the nerve fibers, and concluded that the nerve terminations are too few to reach every muscle cell. Dahl ('16), whose account influenced much of the subsequent discussion of this subject in text books, used methylene blue on human tissue. He stated that the delicate unmyelinated terminal fibers are chiefly distributed parallel with the muscle bundles and end as fine twigs without specific association with individual muscle cells. Comparing his illustrations with our own findings, it is clear that he did not see the terminal picture in its full richness.

Mabuchi ('24), who also studied human material, cautiously described nerves ending freely and also with small spindle-shaped, knobbed or varicose terminal enlargements. Fleming ('28) saw nerve fibers in the myometrium ending in knobs as described by Gavronsky, Köstlin and Mabuchi, and others ending sharply as described by Labhardt. Vinos ('41), using the Golgi method with benefit of the extensive experience of the Cajal Institute, wrote that the unmyelinated nerves form plexuses about and within the fascicles of the muscle. Penetrating into the interior of the muscle bundles, they branch and terminate in fine pointed endings. His picture from the dog's uterus seems to show these terminations on or close to the surface of the muscle cells, but the latter are not clearly depicted. Koppen ('50) studied human operative material, using a modified Bielschowsky silver stain, expressly declaring that with methylene blue he could not stain the finest terminal twigs. He states that the large bundles of originally myelinated fibers branch repeatedly; and losing

their sheaths, finally enter a terminal reticulum which forms the ultimate link between nerve and muscle. "The strands of this reticulum, arranged like a rope-ladder, consist of an exceedingly fine congeries of neurofibrils, which wind about the muscle bundles in a terminal syncytium without beginning and without end." In this description of an anastomotic network of neurofibrils, unlike anything which has previously been reported by investigators of the myometrial nerves, Koppen associates himself with a special concept of the terminal structure of the peripheral nervous system, recently developed by Stöhr, Jr., and his school. A somewhat similar description and interpretation has recently been published by Jabonero ('53), who used the pyridine silver method.

As already stated, the illustrations accompanying all these accounts are unsatisfactory. Evidently the investigators have been able to make only imperfect or fleeting observations. The nerve endings either could not be stained completely or, if well stained by methylene blue, could not be studied under conditions favorable to clear observation and to photographic illustration. All these accounts of the general nature of the nerve terminations are, however, consistent in describing a rather diffuse unspecific type of innervation of the myometrium.

A few published reports of more elaborate and specific neuromuscular and sensory endings, based on metallic impregnations, have never been confirmed by use of methylene blue and need not be reviewed here.

It is generally agreed by the writers we have cited, who studied the human, rabbit and dog uterus, and also by Fleming ('27), who examined the rat, mouse and guinea pig, that the intrinsic myometrial nerve plexus does not contain ganglion cells. Reports to the contrary seem to be based on misinterpretation of metallic impregnations, or on the occasional finding, particularly in human uteri, of ganglionic tissue belonging to the utero-vaginal plexus which has become included within the outer muscular layers of the cervical portion of the uterus. Vinos states that he found ganglion cells of this

kind in the uteri of human infants but not of adults. Davis ('33), who gives a detailed summary of the conflicting older reports on this particular question, found ganglion cells within the musculature of human uteri only in the sub-epithelial plexus of the vaginal portion of the cervix.

MATERIAL AND METHODS

In the research to be described we used in all 24 female rabbits of the Copenhagen and Dutch breeds, 4 to 6 months of age. The more successful of our efforts to demonstrate the myometrial nerves were achieved by the use of methylene blue preceded by local injections of hyaluronidase. The use of this enzyme, which will be explained in detail by Weddell and and Pallie in a forthcoming paper ('53), has been developed on the basis of recent evidence that the spreading of various substances (e.g., toxins of infectious organisms, or solutions introduced by experimental procedures) through the tissues is impeded by viscous intercellular materials such as hyaluronic acid. Treatment with hyaluronidase, in order to decrease the resistance to spreading, has proved useful in demonstrating the nerves of the skin with methylene blue. While this present study was in progress, McGregor ('53) independently advocated the use of hyaluronidase. In the case of the uterus, in which (as implied by all the authors cited above and confirmed by our own experience) the spread of fluids through the muscular tissue is extremely poor, there may be another more specific reason for using hyaluronidase. There is some reason to suppose that the accumulation of hyaluronic acid in the tissues generally is enhanced by the action of estrogen. The reproductive organs being especially dominated by estrogen, they may be even more subject to intercellular blocking by hyaluronic acid than are other tissues, e.g., the skin, in which the rate of spread has chiefly been studied. A summary of recent work relevant to this conjecture is to be found in the symposium of which the paper of Duran-Reynals, Bunting and van Wagenen ('53) is a

part. If this idea is valid, the degree of resistance of the myometrium to the spread of staining fluids would no doubt vary with respect to the ovarian cycle. A few casual observations made in the earlier part of our work, which were in line with such an expectation, have not yet been followed up because the use of hyaluronidase was found to increase so greatly the area of spread of methylene blue injected by local puncture that, for the purpose in hand, any cyclic variation of resistance could be neglected.

Procedure

Under anaesthesia the uterine horns were exposed. As large a portion of the organ as possible was treated with hyaluronidase by local injection with a fine needle just under the peritoneal coat or into the myometrium. The enzyme preparation used was Hyalase (Benger). It was used in a strength of 1000 Benger units dissolved in 5 cm^3 of normal Na Cl. After waiting 15 minutes, during which the uterine horns were lightly massaged to promote spread of the enzyme, methylene blue (methylthionine chloride; Merck) was used in either of two ways:

(a) By local puncture, using 0.02% solution of the dye in normal Na Cl. This is the method successfully introduced by Weddell ('41) for study of the nerve supply of the skin. The needle is inserted into the myometrium and light pressure applied until the spreading of the dye solution ceases.

(b) By injection through the arteries. A cannula is inserted into the left renal or the inferior mesenteric artery near its origin, in the direction of the aorta. The femoral arteries are ligated to limit the vascular bed and thus facilitate flow through the uterus. The aorta is temporarily clamped above the level of origin of the cannulated artery. With a large syringe the solution (methylene blue 0.04%, procaine 1% in normal Na Cl) is injected until widespread staining of the uterus is observed. The blue color, however, tends to blanch almost at once. Finally, the clamp is removed from the aorta

so that circulating blood may flow through the uterus for about 15 to 20 minutes in order to wash the dye solution from the capillaries.

After staining by either method, the uterus is removed and slit longitudinally along the mesometrial insertion. The endometrium is stripped or scraped off. Subsequent fixation with ammonium molybdate follows the procedure outlined by Weddell ('41).

OBSERVATIONS

The methods just described yielded satisfactory staining of the nerves which could be examined over wide areas by direct observation. In preparing photographic records, however, we were handicapped by the necessity of studying thick preparations. Dissection distorts or breaks up the finer branches. Oil immersion lenses cannot be used on undistorted preparations. For this reason, the illustrations are not as lucid as we should wish. The terminations, moreover, are so very delicate that even when well stained they are difficult to photograph and to reproduce by the half-tone process. The larger intrinsic nerves of the uterus are made up of plexiform strands (figs. 1–4) through which course the individual fibers. Each strand consists of finely myelinated and unmyelinated fibers numbering from about a dozen down to two or three, invested by Schwann cells. No ganglion cells are present in this plexus. At intervals the strands divide into smaller strands which ultimately give off single fibers (fig. 10). The latter often, but not everywhere, run parallel with the muscle bundles (fig. 7). The terminal fibers branch separately (fig. 9) and mostly end as naked finely-pointed twigs between the muscle cells (figs. 5–7). We have also not infrequently observed them to end in small terminal enlargements.

The exact relation of the nerve endings to the individual muscle cells still remains somewhat obscure in our preparations. There is at present no technique which can yield a clear picture of both elements at once. Methylene blue when successfully applied renders the finest nerve terminations

visible practically without alteration, as they are in life, as was proved by Weddell and Zander ('50) in their study of the corneal nerves; but the detail is not preserved if the stained myometrium is subjected to dissection or the long preparation necessary for sectioning. The methods employing metallic impregnation (Golgi, Cajal, Bielschowsky) do not reliably indicate the full richness of the finest fibers.

We can only say at present, therefore, that the nerves of the myometrium apparently do not end in an intimate fixed relation to the individual muscle cells. The terminations, both of the pointed and the bulbar varieties, appear to lie in the interstices between the myometrial elements. In muscular tissue so closely packed, this location of course involves close spacial approximation of nerve fibers and muscle cells but in no way suggests a specific nerve-muscle connection such as exists in the motor end plates of skeletal muscle.

Although the number of nerve terminations in proportion to muscular elements seems greater in our preparations than in those of previous workers cited above, we also think as most of them did that the nerve terminations are too few to provide each individual muscle cell with a nerve ending.

The blood vessels in the myometrium are accompanied by unmyelinated nerves resembling those of the muscle layer proper (figs. 11, 12). In the muscular coats of the larger vessels the same manner of nerve termination can be observed as among the uterine muscle.

Immediately under the peritoneal covering of the uterine horns there is a plexus of nerves with endings of the same types as in the myometrium (fig. 8).

DISCUSSION

From observations by Weddell and various co-workers on the nerve supply of the skin, blood vessels and cornea, a general interpretation of the nature of nerve endings is emerging and will be set forth more fully in a forthcoming paper by Weddell and Pallie ('53). It is suggested that all nerves, where they enter into physiological relation with the inner-

vated tissues, terminate in very fine naked filaments. The branching of these end-filaments is of varied extent and complexity, ranging from a wide and diffuse distribution to short and highly circumscribed ramifications such as those of the motor end plates of skeletal muscle and of the Pacinian corpuscles. Whatever the detailed pattern, however, it is thought that the physiological process occurring at the unsheathed ending is fundamentally the same. In the case of efferent impulses a physico-chemical change is produced in the surrounding tissue which sets off the physiological activity of that tissue. At the motor end plates, for example, a specific effect, limited to a single muscle fiber by reason of the anatomical structure, results from the nerve impulse. Where the ramification of the naked terminals is diffusely related to the functioning tissue (as in the blood vessel musculature), widespread motor activity, not very precisely localized or controlled, will take place. In the case of efferent impulses it is similarly assumed that the exposed terminal nerve twigs are affected by changes in their environment which may be of diffuse character, or may be localized in special end-organs. The specificity of nerve action is thus ascribed to the structure innervated, which may be the functioning element alone, e.g., smooth muscle in the blood vessel wall, or the functioning element jointly with a special mediating substance, e.g., the foot plate of the motor ending on striated muscle.

Our observations, reported above, indicate that the manner of innervation of the myometrium is much like that of the blood vessels. The naked filaments are long, branch rather profusely, and end in close association with the contractile elements but certainly without anything more specific in the way of an end-organ than a terminal button and apparently without any such topographical relation of nerve to cell as might be supposed to direct nerve impulses to individual muscle cells. If it be asked whether such observations and interpretations as we are presenting here are compatible with what is known about the rôle of the nervous system in uterine motility, it must be replied that the latter subject is too little

understood to permit more than a conjectural statement. The one established fact is that the uterus can experimentally be made to contract by stimulation of the hypogastric nerves. This means, of course, that some at least of the nerve fibers of the myometrium are to be classified as motor; but what part they play in the physiological activity of the organ as a whole is obscure.

To avoid detailed citation of the extensive and confusing literature, we quote here from a review of what has been reported about the motility of the rabbit's uterus (Reynolds, '49, chapter 2). At the time of estrus, when the estrogenic hormone of the ovary is dominant over the reproductive tract, fairly regular contraction waves commence at the tubal end of the uterine horn in continuation of tubal waves and sweep slowly over the length of the horn, dying out in the region of the cervix. In the rabbit, the contraction waves are not simultaneous in the two horns. If the horn is cut across, there appears to be a greater frequency of contraction in the upper (tubal) portion; this points to a physiological gradient which tends in the intact uterus to direct the contraction waves toward the cervix. Bozler ('37) has pointed out that contractions of the excised myometrium, initiated by direct electrical stimulation, spread through the musculature in a way physiologically characteristic of a syncytium, i.e., the stimulus seems to pass directly from one muscle cell to the next.

At the time of ovulation and for a few days thereafter the motility of the uterus is reduced, presumably under the influence of progesterone. In the anestrous phase and after öophorectomy it is reported that contractions may begin in any part of the uterus and spread in either direction, not however travelling very far. In the latter half of pregnancy, local contraction waves tend to originate at the sites of implanted fetuses, but their spread is only local. As gestation advances the contractions become stronger. During labor there are mild peristaltic contractions of the whole uterus, but evacuation of the fetuses results from a relatively orderly process of local contraction of the successive implantation

sacs, beginning at the cervical end. As for the significance of the movements of the uterus in its function as an organ, there are two phases which seem to require at least an elementary sort of coordination between segments of the uterine horns, namely, the transport and spacing of the embryos before implantation, and parturition. In spite of numerous investigations (cf. Reynolds, '49) we have no clear understanding of the rôle of the myometrial nerves in these two functional activities. It must be re-emphasized that both of them have been reported to occur in denervated uteri and that parturition takes place in orderly fashion even after the uterus is removed from the body; and thus, if nerve impulses are essential to the process, the path of conduction does not necessarily extend beyond the parametrial ganglia of the cervical region. The myometrium itself exhibits (1) spontaneous rhythmic contractility, (2) control of contractile force and rate by estrogen and progesterone, and (3) conduction of the contraction-stimulus by the muscle cells. In view of the clear indication given by recent research (Corner and Csapo, '53) that the ovarian hormones act, in part at least, by altering the permeability of the myometrium, e.g., to potassium ions, it is probable that certain features of the correlated activity at the critical stages of gestation may be controlled by hormone action. For example, the enhanced spread of contraction waves so that they build up into a kind of peristaltic traverse of the whole uterine horn, such as occurs at estrus and in the late stages of gestation, may result, not from coordination by nervous connections, but from enhanced spread of myogenic impulses spreading through the muscle. The observed cyclic variation of the extent of spread of spontaneous contraction waves in the non-pregnant uterus favors this suggestion.

In consideration of these inherent properties and probably also the existence of a gradient of muscular activity along the horns, it seems probable that neural coordination, if required for the more elaborate motility patterns, must be of the sort that could be mediated by innervation of the very primitive

type such as we, in conformity with several previous workers, have described in this paper. An intrinsic mechanism of this sort could convey to the uterus as a whole impulses of central origin coming through the parametrial ganglia and thus could provide for somatic influences upon the uterus, such for example as the often-cited uterine cramps produced by suckling of the newborn infant. It could also respond, within the uterus, to local stimulation brought about by myogenic activity, conveying nerve impulses thus initiated to adjacent regions of the muscle tissue. Because nerve impulses reaching the contractile tissue over non-specific nerve endings of this sort would presumably function by creating physicochemical alterations of the environment or surface of the muscle cells, nerve action could either initiate contraction (as obviously happens when the hypogastric nerves are stimulated), or it could reinforce spontaneous contractility. This sort of generalized, broadly coordinative, nervous action is suggested by Weddell and Pallie ('53) to be operative in the blood vessels. In the case of the uterus, the resulting motor activity would be controlled or modified by endocrine factors, the specific response differing in different hormone states, as in the "pregnancy reversal" first described by Cushny and by Dale ('06). Unless further research, which will probably require the discovery of new technical methods, adds unexpectedly to our present morphological information, nothing more elaborate in the way of nervous control can be safely assumed to operate in the control of uterine function.

Two remaining questions should be mentioned here because, even though further study is required to solve them, our morphological description must be judged as to its compatability with what is known on these topics. Certain investigators, influenced by knowledge of double innervation of some involuntary muscular tissue by fibers of both sympathetic and parasympathetic origin, (as is clearly true, for example, of the iris), have interpreted their experiments on the uterus as indicating a similar dual innervation there. Reynolds ('49), after a careful review of the literature, was,

however, not convinced that parasympathetic fibers innervate the uterine cornua or the fundus of the unicameral uterus. Sauter ('48) in a paper which appeared too late for consideration by Reynolds, states on the basis of pharmacological experiments that the whole uterus receives nervous impulses by way of both "vegetative systems," stimulation of the sympathetic increasing uterine tonus, of the parasympathetic decreasing tonus. Our observations do not of course contribute anything directly to the discussion of this point: but it is becoming clear from biochemical and physiological studies (Corner and Csapo, '53) that all such investigations must henceforth include control of the hormonal state under which the uterus is working at the time of the experiment. The pattern of response of the muscle cells to direct electrical stimulation is very greatly modified by the action of estrogen and progesterone respectively. Csapo (personal communication) informs us that strips of rabbit's uterine muscle, studied isometrically in vitro, will respond to the action of pituitrin in critical concentration by contraction or relaxation according to whichever of these hormones has been administered previous to the experiment. In the light of this information, the evidence that has been put forward for inhibitory or relaxative action on the uterine musculature via parasympathetic nerves is inconclusive. It should be added that investigators with extensive experience of electrical stimulation of the pelvic trunks (e.g., Langley and Anderson, 1895; Rudolph and Ivy, '30; Reynolds and Kaminester, '35) mention only contraction as the result of stimulating the hypogastrics and have obtained no muscular response from stimulation of the pelvic nerves, by which parasympathetic fibers, if present, would be expected to reach the myometrium.

The other question is that concerning the possible existence of special pathways from the uterus to the central nervous system, conveying afferent impulses. Presacral nerve section has been undertaken to relieve painful conditions associated clinically with the uterus, but there is after all no certain proof that the pain of dysmenorrhea and allied morbid af-

fections of the pelvic region actually originates in the uterine muscular tissue. Labate and Reynolds ('37) traced afferent pathways from the ovary and tube experimentally in the cat by observing reflex changes in carotid blood pressure and respiratory movements, elicited by stimulation at various points along the uterine nerve supply. In unpublished work (cited by Reynolds, '49) they traced an afferent path from the uterine cornua of the cat via the hypogastric nerves. A few neurohistologists have reported the existence of supposed sensory endings in the myometrium, the latest of these being Keiffer ('32), who prepared human material by a modification of the Bielschowsky method using excessively strong silver nitrate solutions. We have seen nothing suggesting special sensory end-organs and conclude that afferent impulses from the uterine cornua must originate in the simple nervous structure which we have described in this paper. The precise starting points of sensory impulses may be, for all we know, in any one of the three distinguishable sites; that is to say, in the terminations of the myometrial plexus, of the subperitoneal plexus, and of the plexuses in the walls of the blood vessels.

SUMMARY

The nerve supply of the myometrium of the uterine horns of the rabbit consists of a plexus of finely myelinated and unmyelinated fibers. From this, single fibers are given off which branch and mostly end as finely-pointed twigs between the muscle cells. Some of the twigs end in small terminal enlargements. The nerve endings, although closely approximated to the muscle elements, do not have an intimate fixed relation to individual muscle cells. The terminations are not sufficiently numerous to provide each muscle cell with a nerve ending.

A similar plexus occurs immediately under the peritoneal covering of the uterine horns. The blood vessels in the myometrium are accompanied by unmyelinated nerves with terminations similar to those of the myometrium.

No ganglion cells and no specific sensory endings are found in the uterine horns.

In structure, and presumably in function, the innervation of the myometrium resembles that of the blood vessels. It is suggested that this very primitive type of nerve supply is adequate to mediate the neural control of the uterine muscle, which (so far as understood at present) is broadly coordinative, slow-acting, not sharply localized; and is nonspecific in that the motor response to nervous stimulation is conditioned by the dominant ovarian hormone.

LITERATURE CITED

BACQ, Z. M. 1932 Effect of sympathectomy on sexual functions, lactation, and maternal behavior of the albino rat, with a description of the technique of sympathectomy. Am. J. Physiol., *99:* 444–453.

BOZLER, E. 1937 Physiological evidence for the syncytial character of smooth muscle. Science, *86:* 476.

CANNON, W. B., H. F. NEWTON, E. M. BRIGHT, V. MENKIN AND R. M. MOORE 1929 Some aspects of the physiology of animals surviving complete exclusion of sympathetic nerve fibers. Am. J. Physiol., *89:* 84–107.

CORNER, G. W., AND A. CSAPO 1953 Action of the ovarian hormones on uterine muscle. Brit. Med. J., No. 4812, March *28:* 687–693.

DALE, H. H. 1906 On some physiological actions of ergot. J. Physiol., *34:* 163–206.

DAHL, W. 1916 Die Innervation der weiblichen Genitalien. Ztschr. f. Geburtsh. u. Gynäk., *78:* 539–600.

DAVIS, A. A. 1933 The innervation of the uterus. J. Obst. Gyn. Brit. Emp., *40:* 481–497.

DURAN-REYNALS, F., H. BUNTING AND G. VAN WAGENEN 1950 Studies on the sex skin of *Macaca mulatta*. In: The ground substance of the mesenchyme and hyaluronidase. Ann. N. Y. Acad. Sci., *52:* 1006–1014.

FLEMING, A. W. 1927 The peripheral innervation of the uterus. Trans. Roy. Soc. Edinb., *55:* 507–529.

———— 1928 The intrinsic nervous mechanism of the uterus. J. Obst. Gyn. Brit. Emp., *35:* 247–257.

GOLTZ, F., AND A. FREUSBERG 1874 Ueber den Einfluss des Nervensystems auf die Vorgänge während der Schwangerschaft und der Geburtsakt. Arch. f. d. ges. Physiol., *9:* 552–565.

JARONKO, V. 1953 Etudes sur le système neurovégétative périphérique. VII. Le syncytium nerveux intramural de l'utérus humain. Acta. Anat., *18:* 295–325.

KEIFFER, H. 1932 Le système nerveux végétatif de l'utérus humain. Troisième communication. Bull. Acad. roy. Méd. Belgique, *12:* 719–734.

KILIAN, F. M. 1851 Die Nerven des Uterus. Ztschr. f. rationelle Med., *10:* 41–100.

KOPPEN, K. 1950 Histologische Untersuchungsergebnisse von die Nervenversorgung des Uterus. Arch. f. Gynäk., *177:* 354–391.

LABATE, J. S., AND S. R. M. REYNOLDS 1937 Sensory pathways of the ovarian plexus. Am. J. Obst. Gyn., *34:* 1–11.

LABHARDT, A. 1906 Das Verhalten der Nerven in der Substanz des Uterus. Arch. f. Gynäk., *80:* 135–211.

LANGLEY, J. N., AND H. K. ANDERSON 1895 The innervation of the pelvic and adjoining viscera. J. Physiol., *19:* 71–139.

———— 1896 The innervation of the pelvic and adjoining viscera. J. Physiol., *20:* 372–406.

MABUCHI, K. 1924 Morphologische Studien über das Verhalten der Nerven in den weiblichen Geschlechtsorganen des Menschen mit besonderer Rücksichtigung der Veränderungen ihres Verhaltens während der Gravidität und Menstruation und in zunehmenden Alter. Mitt. a. d. Med. Fak. Kais. Univ., Tokyo, *31:* 385–495.

MCGREGOR, I. A. 1953 Hyaluronidase as an adjuvant to methylene blue in staining of nerve fibres. Nature, *171:* 1070.

REYNOLDS, S. R. M. 1949 Physiology of the Uterus. 2nd Ed.

REYNOLDS, S. R. M., AND S. KAMINESTER 1935 The peripheral motor sympathetic innervation to and within the uterus. Am. J. Physiol., *112:* 640–648.

RUDOLPH, L., AND A. C. IVY 1930 The physiology of the uterus in labor; experimental study of the dog and rabbit. Am. J. Obst. Gyn., *19:* 317–335.

SAUTER, H. 1948 Beitrag zum Problem der Innervation des Uterus. Schw. Med. Wchnschr., *78:* 512–517.

VINOS, A. R. 1941 Sobre el sistema nervioso del utero (inervación intramural). Trab. Inst. Cajal Invest. Biol., *33:* 119–163.

WEDDELL, G. 1941 The pattern of cutaneous innervation in relation to cutaneous sensibility. J. Anat., *75:* 346–367.

WEDDELL, G., AND W. PALLIE 1953 (In preparation.)

WEDDELL, G., AND E. ZANDER 1950 A critical evaluation of methods used to demonstrate tissue neural elements, illustrated by reference to the cornea. J. Anat., Lond., *84:* 168–195.

PLATES

PLATE 1

EXPLANATION OF FIGURES

All photographs are from methylene blue preparations

1 Plexiform arrangement of nerve fibers giving rise to fine naked axoplasmic terminals which end freely in the myometrium. × 210.

2 Bundle of nerve fibers under higher magnification, in which the axis cylinders have been selectively stained by methylene blue. × 400.

3 Similar but more deeply stained bundles. × 300.

4 Bundle of nerve fibers showing (arrow) location of a node of Ranvier. × 210.

5 Fine naked axoplasmic terminals ending in the myometrium. The muscle cells are just distinguishable and some blood cells in capillaries have been stained. × 360.

6 Part of area shown in figure 5, under higher magnification. The terminal filaments are finely beaded in methylene blue preparations. × 720.

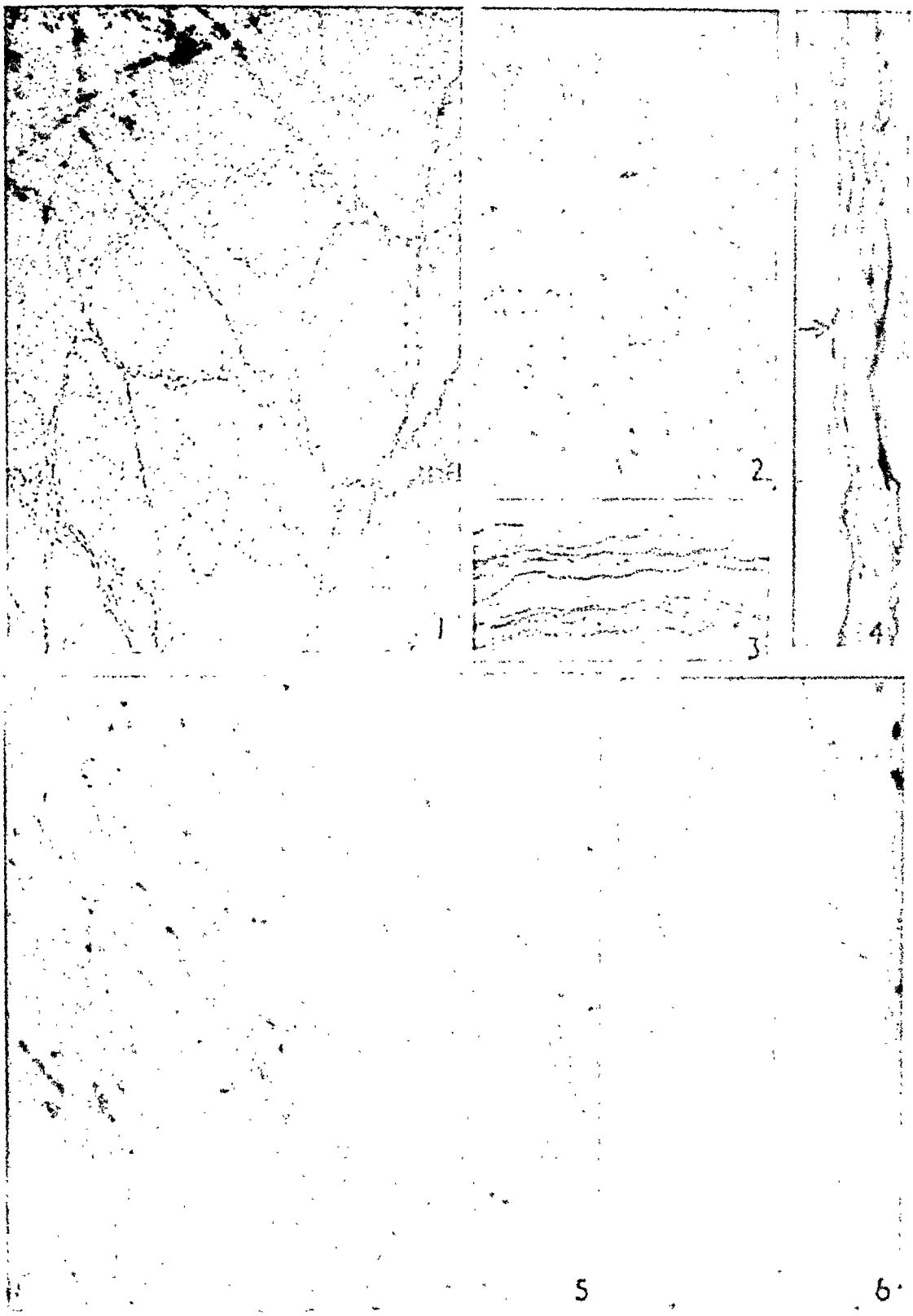
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PLATE 2

EXPLANATION OF FIGURES

All photographs are from methylene blue preparations

7 Fine naked axoplasmic filaments ending in the myometrium. The majority of terminals are running in a direction parallel to the long axis of the muscle fibers. × 430.

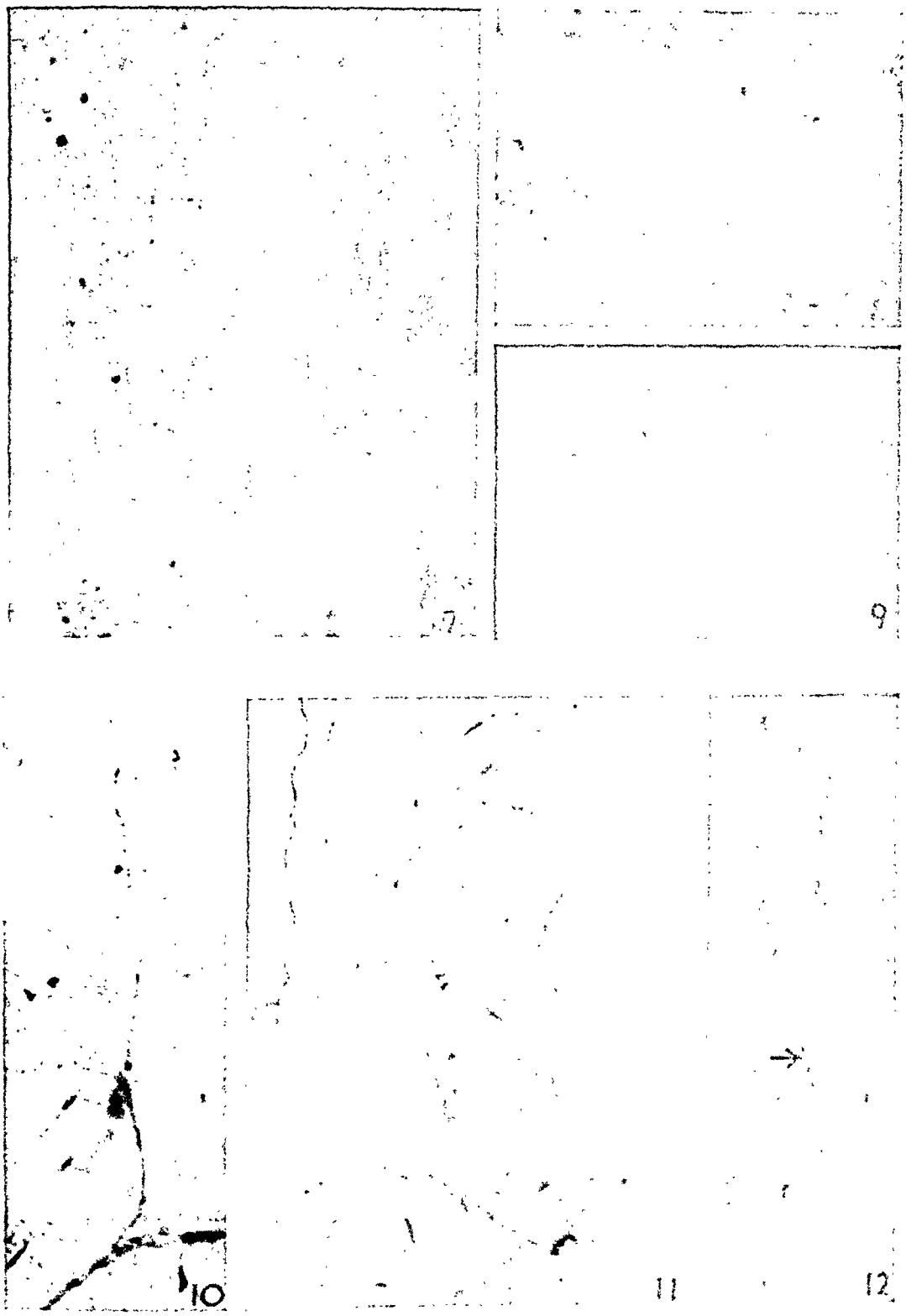
8 Fine naked axoplasmic filaments ending just beneath the peritoneal coat of the uterus. × 850.

9 Fine naked axoplasmic filaments branching repeatedly just before terminating. × 430.

10 Origin from the plexiform strands of stem nerve fibers which give rise to the terminals. × 470.

11 Stem fibers and fine naked axoplasmic terminals ending in relation to an arteriole. A few of the muscle fibers of the vessel wall are faintly stained. × 470.

12 Stem fibers giving rise to naked terminals ending in relation to an arteriole. The arrow points to the stained nucleus of a Schwann cell. Only the finest terminals are entirely naked. × 470.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STUDIES ON SYNOVIAL PERMEABILITY

I. DIRECT MEASUREMENTS OF SYNOVIAL PERMEABILITY IN RATS[1]

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TWO FIGURES

Synovial tissue, which is well vascularized and composed of an intercellular ground substance, connective tissue fibers and modified connective tissue cells, is permeable to fluids and colloids. The reviews by Gardner ('50), Bauer, Ropes and Waine ('40) and Edlund ('49) show that true solutions, colloidal solutions and fine suspensions pass through the synovial tissue rapidly, the rate being inversely proportional to particle size up to 100 μ. Particles larger than this do not diffuse through the synovial tissue. According to the above investigators substances composed of large molecules, such as proteins, leave the joint cavity by diffusing through the synovial tissue and into the lymphatics whereas the small molecule substances like diffusible dyes are removed by both the lymphatics and the synovial capillaries. Steck, Joseph and Reed ('48) demonstrated, by measuring the pH of synovial fluid in the dog, that acid metabolites from muscle activity are transferred directly and rapidly to the joint cavity by way of the tissue fluid without the mediation of the capillary or lymphatic circulation.

From the information provided by the above references, synovial permeability can be defined as the capacity of synovial

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tissue to allow the passage of fluids and colloids in either direction between the joint cavity and the synovial blood vessels, lymphatics and surrounding tissue.

Most of the recent studies on synovial permeability have been clinical ones which attempt to explain the marked improvement of arthritic patients in response to adrenocorticosteroid therapy. Kling ('38) and Maunsell et al. ('50) found that changes in synovial permeability are associated with joint pathology and Efskind ('43) states that such changes can be detected earlier than histological alterations as signs of pathology. Basic information such as the effects of age, sex, hormones, diet, etc., on synovial permeability is incomplete.

The current method for measuring synovial permeability as described by Seifter et al. ('49) involves the renal excretion of phenolsulfonphthalein from the blood after the dye has been injected into the joint cavity. This technique has been used on rabbits and humans but it is not considered adequate for experimental work on rats because it does not provide information concerning the manner in which permeability is changed and it is not suitable for use on small laboratory animals.

The two methods of measuring synovial permeability to be described in this paper satisfy the above criteria and in addition they are based on direct observation of the synovial joint.

The author expresses sincere appreciation for the guidance and suggestions of Dr. Earl O. Butcher during this investigation.

MATERIAL AND METHODS

Wistar albino rats of both sexes, about three months old, were anesthetized with Nembutal. The hair was then shaved from the skin covering the knee joint and the animal was placed supinely with one leg extended and immobilized. The following commercial radiopaque solutions were injected into the extended knee joints to see which preparation was most suitable: Diodrast, a 35% aqueous solution of an organic iodine salt; Thorotrast, an aqueous suspension (concentration un-

known) of thorium dioxide, and Neo-Iopax, a 50% or 75% aqueous solution of sodium iodomethamate. The injection apparatus consisted of a 1 cm^3 tuberculin syringe, calibrated into .01 cm^3 and connected by a length of no. 7 French rubber catheter to a short-beveled 21 gauge hypodermic needle (fig. 1). Beebe loupe magnifying glasses were used for estimating the syringe calibrations to thousandths of a cubic centimeter. The injection of radiopaque fluid into the knee joint cavity was observed by means of a dental x-ray machine and dental films.

After the syringe plunger was moistened with water to reduce the friction between it and the barrel, the syringe, with catheter and needle attached, was filled to .400 cm^3 with the radiopaque solution. The catheter was then clamped shut and the syringe mounted vertically with its lower end on the same horizontal plane as the joint to be injected. The needle was inserted into the knee joint cavity just inferior to the patella and its location was checked by a radiogram. A 1, 5, or 10 gm weight was placed on the plunger; the catheter was unclamped and the joint cavity allowed to fill with the solution. If leakage occurred or the joint did not fill completely, as shown by a radiogram, the procedure was repeated on another knee.

OBSERVATIONS

Radiopaque solutions

A radiopaque solution was desired which would diffuse readily through the synovial tissue. Diodrast and Neo-Iopax both satisfied this qualification but occasionally an injection of Neo-Iopax caused flexion of the limb and an increased respiratory rate in the anesthetized animal. These symptoms were not seen when Diodrast was used, therefore all injections reported in the data were made with this solution. The Thorotrast suspension was of no value because it did not diffuse through the synovial tissue. Radiograms taken three days after the Thorotrast was injected showed it still visible in the joint cavity.

Injection pressure

A 5 gm weight mounted on the plunger produced an injection pressure of approximately 58 cm of water and all injections reported here were made at this pressure. Other size weights were found unsatisfactory for various reasons. The 1 gm weight was not heavy enough to give a reproducible injection pressure because the friction between the plunger and the barrel would often interfere with or stop the injection. This frictional variation was reduced to a relatively insignificant factor by using the 5 gm weight. The 10 gm weight was too heavy because on rare occasions it ruptured the joint capsule and the entire contents of the syringe rapidly spread out along the fascial planes of the leg. In this respect also, the 5 gm weight was preferable because it produced an injection pressure which was considerably less than that required to rupture the joint capsule.

The height of the syringe in relation to the knee joint was changed in several injections to note its effect on the injection pressure as shown by a corresponding change in the rate of Diodrast flow through the joint cavity. In one instance the flow was doubled by elevating the syringe 4 cm. It was arbitrarily decided to keep the lower end of the syringe on a level with the knee joint for all injections.

Quantity of Diodrast and time required to fill the knee joint

Diodrast entered each knee joint cavity rapidly and filled it during the first 15 seconds as shown by a radiogram taken at the end of this time. The injection was continued for one minute, however, in order to allow a brief period for stabilization of the intraarticular pressure before measuring the removal time or diffusion rate. In 19 rats an average of $.091 \pm .016$ cm^3 of Diodrast entered the knee joint in one minute. This quantity represents the approximate size of the joint cavity because only .003 cm^3 entered the joint during the 45 second stabilization period.

Diodrast removal time

When the knee joint cavity was filled with Diodrast and subsequently x-rayed, the radiograms showed the Diodrast entering the synovial tissue where it was removed mainly by the blood stream. The number of minutes required, after the start of the injection, for the Diodrast to be completely removed from the joint cavity and synovial tissue — as evidenced by its disappearance in the final radiogram — was referred to as the Diodrast removal time.

This observation was made on 24 rats by allowing Diodrast to flow into the knee joint cavity for one minute. The injection was stopped by clamping the catheter and the needle was left inserted into the joint cavity to prevent leakage. Radiograms of the joint, taken from the medial aspect at approximately 5 minute intervals, showed the average removal time to be 30 ± 1.5 minutes. The radiograms in figure 2 represent three stages in the injection and diffusion of Diodrast during a removal time determination.

Diodrast diffusion rate

In another set of experiments Diodrast was injected into the knee joint cavity for as long as 15 minutes. After the cavity was filled, which required less than one minute, the solution continued to enter the joint at a slower injection rate until the catheter was clamped shut. The Diodrast diffusing through the synovial tissue was apparently being replaced by the continuous flow from the syringe, therefore, the quantity which entered the filled joint during a definite time interval was recorded as the Diodrast diffusion [3] rate in the following manner.

Diodrast was injected continuously for 15 minutes into each of 22 knee joints. Syringe readings were made at 1, 5, 10 and 15 minutes after the start of the injection and radiograms were taken at the first and last readings to check for complete filling of the joint cavity. After the first minute was allowed for the joint to fill completely, the quantity of Diodrast which

[3] The term diffusion is used in reference to an isotonic type of diffusion.

entered the joint during the next 14 minutes was recorded as the diffusion rate, the mean value of which was .064 ± .007 cm^3 per 14 minutes (table 1). Converting the readings to cubic centimeters per minute gave values too small to be read on the syringe calibrations.

TABLE 1

Diodrast diffusion rates in knee joints of 22 normal rats, approximately three months old

Cm^3 of Diodrast diffusing through knee joint

AGE	SEX	FIRST PERIOD *(2–5 min.)*	SECOND PERIOD *(6–10 min.)*	THIRD PERIOD *(11–15 min.)*	TOTAL *(14 min.)*
(days)					
91	m	.019	.024	.027	.070
90	m	.019	.029	.030	.078
79	m	0.16	.025	.020	.061
79	m	.020	.034	.019	.073
102	m	.012	.011	.027	.050
84	m	.012	.026	.027	.065
90	m	.021	.020	.028	.069
80	m	.020	.020	.025	.065
94	m	.020	.026	.022	.068
95	m	.020	.016	.020	.056
95	m	.013	.019	.026	.058
95	m	.012	.016	.036	.064
95	m	.020	.017	.025	.062
95	m	.013	.025	.019	.057
96	m	.015	.020	.025	.060
96	m	.023	.019	.025	.067
91	f	.020	.019	.026	.065
75	f	.024	.016	.021	.061
75	f	.025	.024	.025	.074
87	f	.017	.022	.028	.067
94	f	.012	.018	.022	.052
96	f	.019	.021	.024	.064
Mean ± S.E.		.018 ± .004	.021 ± .005	.025 ± .004	.064 ± .007

Statistical analysis of diffusion rates

The diffusion rate was not constant during the entire 14 minute injection, which was another reason for not expressing the diffusion rate in terms of the average flow per minute. During the third period of measurement (11 to 15 minutes) the flow was significantly greater than that for the second

period (6 to 10 minutes). The difference between the first and third periods is not significant. Explanations for these differences and for the individual variations seen especially in the second and third periods are not known to the author. There is no significant difference between the diffusion rates of the males and females listed in table 1.

The mean cumulative flow for the first 4 minutes was .018 ± .004 cm^3; for 9 minutes the flow totaled .039 ± .007 cm^3; for 14 minutes it was .064 ± .007 cm^3. The per cent error or standard error divided by the mean, times 100, for each of these values is 22.2%, 17.9% and 10.9% respectively. This continued decrease in the error, relative to its mean, makes the 14 minute reading the most useful one for a comparison of control and experimental groups.

DISCUSSION

The methods described in this paper represent two different approaches to the problem of measuring synovial permeability. One, the removal time method, is an active process in which a limited amount of fluid, injected into the joint, is gradually removed from the cavity and synovial tissue mainly by vascular channels. This method is identical to the one used by Efskind ('43) in his clinical study on human knee joints.

The diffusion rate method, in contrast to the method just described, depends on a passive process, the forced diffusion of fluid through the synovial tissue in response to a continuous intraarticular injection of that fluid. In this method the radiograms serve only as a precautionary measure to make certain that the joint cavity was completely filled during the 14 minute diffusion interval. Both methods, however, offer the advantages of x-ray visualization.

The degree of flexion and the movement of the joint were controlled because the size and intraarticular pressure of the joint cavity vary depending on the position of the joint and whether or not it is bearing weight. Bauer, Ropes and Waine ('40) state that synovial fluid is under a slight negative pressure of from −2 to −12 cm of water depending upon the posi-

tion of the joint. The importance of controlling the injection pressure was demonstrated in the present paper by changing the height of the syringe and observing the marked effect this had on the diffusion rate.

The amount of synovial fluid present in the normal joint cavity is so small that it does not become a significant variable in these methods. Only "one drop" of synovial fluid can be aspirated from the rabbit knee joint (Bauer, Ropes and Waine, '40) even though the cavity has a volume of approximately .85 cm^3 according to Edlund ('49). The rat knee joint cavity is about one-tenth this size and contains a proportionately smaller amount of fluid. When the synovial cavity is opened in the rat one sees only well moistened surfaces and no free fluid.

The Diodrast diffusion rate and removal time methods are being applied to a study of synovial permeability in the rat which will be reported later. These techniques might be advantageously used on other animals in view of the recent reports on synovial permeability which indicate the need for a more reliable method of measurement. Hidalgo et al. ('52) used the kidney function technique but were unable to duplicate Seifter's results. Paul et al. ('52) experienced the same difficulty and in their conclusions they question the validity of this procedure as an accurate measure of synovial permeability.

SUMMARY

Two methods of measuring synovial permeability are described. Both are based on the injection of a radiopaque solution into the knee joint cavity of Wistar albino rats approximately three months old.

In the removal time method, the joint cavity is filled with Diodrast and radiograms are taken until the solution is no longer visible.

The diffusion rate method measures the amount of Diodrast which diffuses through the synovial tissue during a continuous 14 minute intraarticular injection.

The removal time, 30 ± 1.5 minutes, and the diffusion rate, .064 ± .007 cm³ per 14 minutes, are given as control figures for future experimental work.

LITERATURE CITED

BAUER, W., M. W. ROPES AND H. WAINE 1940 The physiology of synovial structures. Physiol. Rev., *20*: 272–312.

EDLUND, J. 1949 Studies on absorption of colloids and fluid from rabbit knee joints. Acta Physiol. Scandinav., *18* (suppl. 62): 1–108.

EFSKIND, L. 1943 Clinical studies on the function of the synovial membrane of the knee joint. Acta Chir. Scandinav., *88*: 37–48.

GARDNER, E. 1950 Physiology of movable joints. Physiol. Rev., *30*: 127–176.

HIDALGO, J., C. D. MCCLURE, J. B. HENDERSON, R. W. WHITEHEAD AND C. J. SMYTH 1952 Effect on joint permeability of adrenal cortex extract, cortisone, hydroxyphenyl cinchoninic acid and hyaluronidase. Proc. Soc. Exp. Biol. and Med., *80*: 97–101.

KLING, D. H. 1938 The Synovial Membrane and the Synovial Fluid. Medical Press, Los Angeles, p. 53.

MAUNSELL, K., F. WRIGLEY, T. C. HIGHTON AND L. B. HOLT 1950 The synovial membrane. Some observations in normal and arthritic human joints. Ann. Rheum. Dis., *9*: 363–371.

PAUL, W. D., R. E. HODGES, R. W. KNOUSE AND C. S. WRIGHT 1952 Effect of anti-rheumatic drugs on synovial membrane permeability. Proc. Soc. Exp. Biol. and Med., *79*: 68–71.

SEIFTER, J., D. BAEDER AND A. BEGANY 1949 Influence of hyaluronidase and steroids on permeability of synovial membrane. Proc. Soc. Exp. Biol. and Med., *72*: 277–282.

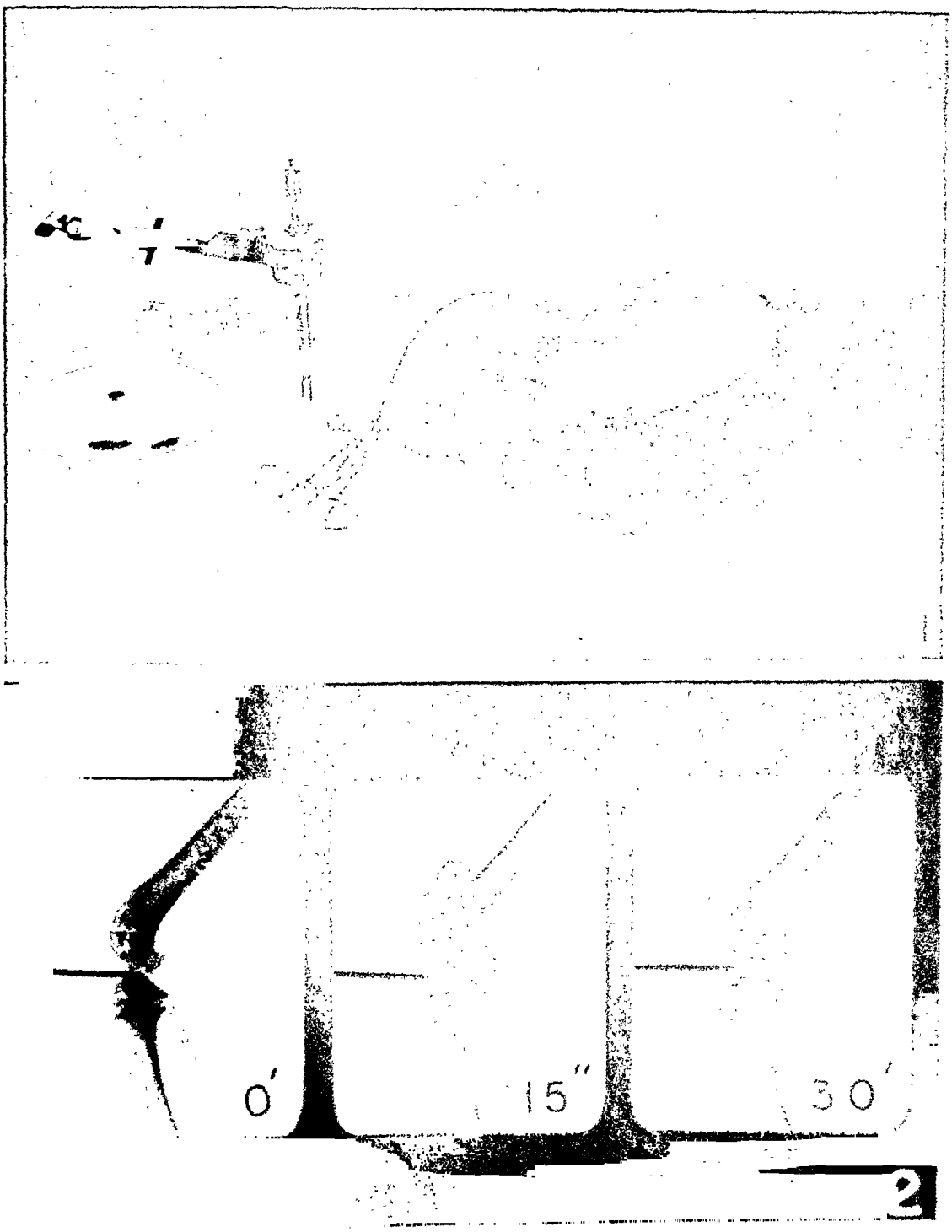
STECK, I. E., N. R. JOSEPH AND C. I. REED 1948 The pH of the synovial fluid in the anesthetized dog under treatment with methazol or insulin. J. Bone and Joint Surg., *30A*: 500–505.

PLATE 1

EXPLANATION OF FIGURES

1 The intraarticular injection apparatus.

2 Radiogram showing the injection of Diodrast into the knee joint cavity of a young rat. × 1.7.
Zero minute film: Needle in joint cavity just before injection.
Fifteen second film: Joint cavity filled with Diodrast.
Thirty minute film: Diodrast already disappeared from joint.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STUDIES ON SYNOVIAL PERMEABILITY

II. FACTORS INFLUENCING SYNOVIAL PERMEABILITY IN THE RAT [1]

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Changes in synovial permeability are currently implicated in the onset and treatment of certain synovial joint diseases (Seifter et al., '49; Maunsell et al., '50; Jaworski et al., '50; Paul et al., '52; Hidalgo et al., '52). There is still a controversy, however, over the significance of such permeability changes, a situation which probably results from the lack of an adequate method for measuring synovial permeability and from a scarcity of information about the permeability alterations which occur normally.

This paper reports the effects of certain intraarticular and systemic variables on synovial permeability in the rat, using two methods of measurement, the Diodrast diffusion rate and removal time methods, which were developed especially for application to small laboratory animals. The objectives of the work reported here are: (1) to determine what information is provided by the diffusion rate and removal time methods on the manner in which synovial permeability is altered; (2) to demonstrate by these methods any normal variations in synovial permeability which might be associated with age and sexual maturity; and (3) to study the

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effect of the adrenal glands and their cortical secretions on synovial permeability in an attempt to explain the action of adrenal hormone therapy on pathological joints.

MATERIAL AND METHODS

Two hundred and sixteen permeability measurements were made on the knee joints of approximately 170 Wistar albino rats of both sexes. The permeability measurements were made by the Diodrast diffusion rate and removal time methods, using an injection apparatus which was described with these techniques in the preceding paper. To summarize them briefly, the Diodrast diffusion rate is obtained by measuring the quantity of Diodrast which diffuses through the knee joint cavity and synovial tissue during a continuous 14 minute intraarticular injection of that solution; the Diodrast removal time represents the number of minutes required for the Diodrast to disappear in radiograms of the knee joint taken at intervals after a one minute injection of that solution.

The rats were classified into the following experimental groups, utilizing when possible, littermate controls (table 1). Unless specified otherwise, all rats used were mature animals approximately three months old.

1. *Rats with an altered synovial ground substance: Groups 1a, 1b, 1c, 1d:*

Testicular hyaluronidase,[3] assayed at 1400 turbidity reducing units per milligram, was used in groups 1b and 1d.

2. *Rats with an altered synovial blood supply: Groups 1a, 1c, 2a, 2b, 2c:*
3. *Rats of different ages*[4]*: Groups 3a, 3b, 3c, 3d, 3e:*

The ages of the rats are given in table 1.

4. *Gonadectomized rats: Groups 4a, 4b:*
5. *Adrenalectomized rats: Groups 5a, 5b, 5c:*

[3] The testicular hyaluronidase was supplied by Dr. J. Seifter of the Wyeth Institute of Applied Biochemistry, Philadelphia, Pennsylvania.

[4] Rats over 18 months of age were made available by Dr. C. M. McCay, New York State College of Agriculture, Cornell University, Ithaca, New York.

6. Adrenalectomized rats treated with cortisone[5]*: Groups 6a, 6b, 6c 6d:*

Cortisone acetate in aqueous suspension (25 mg per cm^3) was used in groups 6b and 6d.

7. Normal rats treated with desoxycorticosterone acetate[6]*: Groups 7a, 7b:*

An aqueous suspension of desoxycorticosterone acetate (75 mg per cm^3) was used.

OBSERVATIONS

Altered synovial ground substance, groups 1a, 1b, 1c, and 1d. Group 1a (table 1) represents control permeability measurements which were made by the Diodrast diffusion rate and removal time methods on one knee joint in each of 12 anesthetized rats. Group 1b consists of 12 experimental permeability measurements made on the other knee of the same animals after the synovial ground substance had been altered. Groups 1c and 1d represent similar control and experimental groups, respectively, except that these measurements were made on 6 rats that had been killed with Nembutal two minutes prior to the permeability determination.

The synovial ground substance was altered in experimental groups 1b and 1d by dissolving 2 mg of testicular hyaluronidase in the first .1 cm^3 of Diodrast to enter the knee joint cavity during the diffusion rate and removal time determinations. The control measurements were obtained by using Diodrast which did not contain hyaluronidase. In half of the rats in groups 1c and 1d, the order in which the control and experimental measurements were made was reversed to make the post-mortem time interval equal in both groups.

The addition of hyaluronidase to the Diodrast caused a greater than two-fold increase in the Diodrast diffusion rate in both live and dead rats. The diffusion rate rose from an

[5] The cortisone acetate used was an aqueous suspension of [illegible] Merck and Co., Rahway, New Jersey.

[6] The desoxycorticosterone acetate was supplied [illegible] Corporation, Bloomfield, New Jersey.

TABLE 1

Effects of age, hormones, etc. on the Diodrast diffusion rate and removal time in rat knee joints

GROUP	ANIMALS	AGE AT TEST	DIFFUSION RATE (cm^3 per 14 minutes)	REMOVAL TIME (minutes)	GROUP COMPARED TO	P
1a	12 controls	3 mos	.063	30		
1b	12 2 mg hyaluronidase	3 mos	.156	30		
1c	6 controls, dead	3 mos	.068	> 60		
1d	6 2 mg hy'dase, dead	3 mos	.140	> 60		
2a	5 controls	3 mos		32		
2b	5 2.5 cc card. punct.	3 mos		55		
2c	3 physiological shock	3 mos		60		
3a	7 normal	28–30 days	.126 ± 0.11			
3b	8 normal	2–3 mos	.062 ± .004		3a	.01
3c	8 normal	20–24 mos	.059 ± .003		3b	.60
3d	13 normal	3–7½ mos		35 ± 1.5		
3e	10 normal	19–24 mos		80 ± 11.0	3d	.01
4a	4 controls	3 mos	.068 ± .011			
4b	13 gonadectomized	3 mos	.067 ± .003		4a	> .8
5a	12 controls	2–8 mos	.062 ± .009			
5b	15 adrenalectomized	2–5 mos	.109 ± .005		5a	.01
5c	15 adrenalectomized	6–8 mos	.072 ± .007		5a	> .2
6a	8 adrenalectomized	2–5 mos	.107 ± .007			
6b	8 adrx. 8–10 mg cort.	2–5 mos	.090 ± .008		6a	> .2
6c	6 adrx. aq. vehicle	2–5 mos	.093 ± .006			
6d	6 adrx. 2.5–4 mg cort.	2–5 mos	.073 ± .002		6c	> .2
7a	10 controls	3 mos	.060 ± .007			
7b	24 2–18 mg DOCA	3 mos	.079 ± .006		7a	> .05

average control value of .063 cm^3 per 14 minutes to .156 cm^3 in the experimental measurements on live rats, with a comparable increase seen also in the dead rats (table 1).

The removal time measurements were unaffected by the addition of hyaluronidase to the Diodrast. The difference which occurred between the removal time measurements in live and dead rats was seen in both the control and experimental groups and therefore is not due to hyaluronidase treatment.

Altered synovial blood supply, groups 1a, 1c, 2a, 2b, and 2c. In addition to their role as controls in experiments on the ground substance, groups 1a and 1c were also analyzed for effects on permeability due to a change in the blood supply of the joint. Group 1a includes 12 live mature rats which served as controls for group 1c in which the 6 animals were killed two minutes prior to the permeability measurements. Synovial permeability was measured in both of these groups by the Diodrast diffusion rate and removal time methods.

Only the removal time method was used in groups 2a, 2b, and 2c. After a control measurement was made on one knee of each rat in group 2a, 2.5 cm^3 of blood was removed by cardiac puncture and a permeability measurement was made on the other knee joint and recorded as group 2b. Group 2c consists of three adrenalectomized rats which had gone into a state of fatal physiological shock during anesthesia.

The Diodrast diffusion rate showed no significant change in dead rats as compared to live controls (groups 1a and 1c).

The Diodrast removal time was significantly prolonged in each of the experimental groups. From a value of 30 minutes in live controls, it was extended to more than 60 minutes in dead rats (group 1c), to 55 minutes in bled rats (group 2b), and to 60 minutes for those rats in shock (group 2c).

Different ages, groups 3a, 3b, 3c, 3d, and 3e. The rats in these groups were all normal animals classified according to age (table 1).

A decrease in the Diodrast diffusion rate was seen in rats between the ages of one and two months. The average diffusion rate in 7 one month old rats (group 3a) was .126 cm^3

per 14 minutes. In the next age group, two to three months (group 3b), the average diffusion rate had already diminished to .062 cm^3 per 14 minutes. No other significant change appeared in the Diodrast diffusion rate through 24 months of age.

The Diodrast removal time was prolonged from a value of 35 minutes in rats three to 7½ months old (group 3d) to 80 minutes in rats 19 to 24 months old (group 3e).

Gonadectomy, groups 4a, 4b. Group 4b includes 7 males and 6 females gonadectomized at the age of one month. The synovial permeability of these rats and of the controls in group 4a was measured by the Diodrast diffusion rate method at the age of three months.

Removal of the testes or ovaries did not alter the Diodrast diffusion rate from that seen in normal rats of the same age. The diffusion rates in the control and gonadectomized groups at the age of three months were .068 cm^3 and .067 cm^3 per 14 minutes respectively.

Adrenalectomy, groups 5a, 5b, and 5c. The 12 rats in 5a are littermate controls of the adrenalectomized animals in groups 5b and 5c. Group 5b consists of 15 rats adrenalectomized at the age of two to 5 months; in 5c there are 15 rats adrenalectomized at an age between 6 and 8 months.

Measurements of the Diodrast diffusion rate were made from 5 days to two weeks following the adrenalectomy; during this time the adrenalectomized rats were given 1% NaCl for drinking water. To be considered as completely adrenalectomized, the rats had to show a marked weight loss following the operation and succumb in a few weeks when given only tap water for drinking after the completion of the permeability measurements.

The Diodrast diffusion rate was significantly increased following adrenalectomy in rats two to 5 months old, a change which could be demonstated in live and dead animals as early as 5 days after the operation. When the operation was performed on older rats (group 2c), there was no significant increase in the Diodrast diffusion rate.

Adrenalectomy had no effect on the Diodrast *removal time* in both experimental groups.

Adrenalectomized rats treated with cortisone, groups 6a, 6b, 6c, 6d. The same 8 animals were used for groups 6a and 6b. The Diodrast diffusion rates recorded in 6a were made as control measurements on one knee joint of each of these adrenalectomized animals. After the adrenalectomized rats were given a total of from 8 to 10 mg of cortisone intraperitoneally over a period ranging from one hour to 4 days, the diffusion rate was measured on the other knee and record as group 6b.

Groups 6c and 6d represent diffusion rate measurements which were made on another 6 adrenalectomized animals to test for a local action of cortisone. The diffusion rates in 6d were obtained by adding 2.5 to 4 mg of cortisone in aqueous suspension to the Diodrast used during the permeability measurement. As a control only the aqueous vehicle of the cortisone suspension was added to the Diodrast used in group 6c.

The intraperitoneal injection of cortisone into 8 adrenalectomized rats caused a small, statistically insignificant decrease in the Diodrast diffusion rate when compared to adrenalectomized untreated controls. Even when the cortisone was injected intraarticularly in 6 rats, the decrease in diffusion rate was still insignificant. Those knee joints which received an intraarticular injection of cortisone were dissected following the permeability measurements and the particles of cortisone were still present in the joint cavity.

Normal rats treated with desoxycorticosterone acetate, groups 7a, 7b. The control measurements of the Diodrast diffusion rate were determined in one knee of 10 animals (group 7a). These same animals were then treated with desoxycorticosterone and constitute part of the 24 animals in group 7b. Twenty-one of the rats in group 7b received a total of 2 to 18 mg of desoxycorticosterone acetate intraperitoneally over a period of from one hour to 5 days preceding the experimental permeability measurement. The three remaining rats in group

7b were given 2 mg of desoxycorticosterone acetate intraarticularly with the Diodrast used in measuring the diffusion rate.

A small increase in synovial permeability, on the border line of being statistically significant, was seen in group 7b, the P value being just over .05. The effect of the treatment was the same whether the desoxycorticosterone acetate was injected intraperitoneally or intraarticularly. Extending the treatment over a period of 5 days at a level of 4 mg per day did not cause a greater effect than one such injection given one hour before the permeability measurement.

DISCUSSION

Synovial permeability — means of alteration

Experiments reported in this paper demonstrate that the Diodrast diffusion rate and removal time methods provide specific information on the manner in which synovial permeability is altered. The marked increase in the Diodrast diffusion rate following hyaluronidase treatment is indicative of a change in synovial permeability due specifically to an altered ground substance. The fact that hyaluronidase acts on the synovial ground substance to increase its permeability is well substantiated by Meyer ('50), Bunting ('50), Bensley ('50), and Duran-Reynals et al. ('50). Diodrast diffusion rate measurements are not influenced by changes in the synovial blood supply because the Diodrast is not required to enter the blood vessels in this procedure.

The specificity of the Diodrast diffusion rate method for synovial ground substance alterations is matched by the specificity of the Diodrast removal time method for changes in the synovial blood supply. When the synovial blood supply was altered by three different conditions, death, decreased blood volume and physiological shock, corresponding changes were observed in the removal time. The prolonged removal time seen in the adrenalectomized animals during a state of shock was not caused by the adrenalectomy *per se* because this op-

eration was shown to have no effect on removal time measurements. It is possible, therefore, through the combined use of the Diodrast diffusion rate and removal time methods, to detect changes in synovial permeability and then identify the manner in which those changes were brought about.

Both the ground substance and the blood supply of synovial tissue should be considered as factors influencing synovial permeability because: (1) from an anatomical point of view, both structures, the ground substance and the synovial capillaries, are intrinsic parts of synovial tissue; and (2) considered functionally, synovial permeability is that quality of synovial tissue which affects the passage of fluids and colloids between the joint cavity and the blood stream. Unless a technique is used which indicates the manner of permeability change, reliable conclusions as to the cause and significance of such permeability alterations cannot be made.

The specificity of the Diodrast diffusion rate method for detecting ground substance changes is not seen in those procedures based on the passage of a dye or some other substance from the joint cavity into the blood stream, a process which introduces the variable of circulatory alterations. Judging from the experiments with hyaluronidase reported here and from the work done by Hechter ('50) in his study on the mechanisms of spreading factor action, procedures involving the detection of an indicator in the blood stream after that substance has been deposited in the joint cavity, do not reliably indicate changes in the synovial ground substance.

Besides being subject to circulatory variations, these procedures are affected by mechanical factors involving the injection technique. Hechter showed that merely placing the hyaluronidase in contact with the ground substance does not insure that the enzyme will exert its effect on the ground substance substrate because hyaluronidase apparently diffuses very slowly in the absence of a localized pressure increase. When the hyaluronidase, or the solution which is being used in the permeability measurement, is injected into

the joint cavity in a small quantity causing no increase in intraarticular pressure, or when the intraarticular pressure is not maintained at a higher than normal level by a continuous injection of fluid, the state of the synovial ground substance is not accurately measured. The Diodrast removal time measurements, which were obtained in this way, were found to be unaffected by the addition of hyaluronidase.

When the intraarticular pressure was maintained at a higher than normal level by the continuous injection of fluid, as in the Diodrast diffusion rate procedure, then the effect of the hyaluronidase was readily detected. These conditions explain why hyaluronidase activity can be demonstrated by the Diodrast diffusion rate method and not by the removal time method. The same explanation probably holds true for the conflicting results which different investigators have obtained in measuring synovial permeability by the kidney function technique (Seifter, '49).

The kidney function technique is subject to criticism as a measure of synovial permeability because of the large number of variables which must be controlled and also because of the indirectness of the procedure. Seifter et al. ('52) list 7 critical factors, any one of which is sufficient to alter the results obtained by the kidney function method. The influence of injection pressure is not included in this list. Five out of 7 of these critical factors do not exist when the Diodrast diffusion rate method is used to study the permeability of synovial ground substance: (1) the care of the animal is not critical since the method can be applied to dead or live animals; (2) there is no need for catheterizing the urethra; (3) mechanical restriction of the blood supply to the joint area, (4) vasoconstriction and (5) vasodilation, do not influence the Diodrast diffusion rate measurements. The two remaining factors are easily controlled: (1) injections and comparisons are made only on joints of identical anatomy, in this case the knee joint; (2) the accuracy of injection into the joint cavity without leakage is made certain by the use of a radiopaque solution and x-ray visualization.

Normal variations in synovial permeability

Synovial permeability is altered normally by changes in the synovial ground substance and blood supply at different times during the life span of the rat. A decrease in the Diodrast diffusion rate occurred in rats at the approximate age of two months. Sexual maturation was not responsible for this decrease because the change still appeared in gonadectomized rats. A similar decrease in the permeability of rabbit connective tissue was reported by Duran-Reynals ('42) who observed that the intradermal spreading of India ink in rabbits one to two years old was much less than that seen in their 25 to 60 day old progeny. The observation by Lurie and Zappasodi ('39) that the adult type of permeability appears in the skin of rabbits by the age of three months, with no further change occurring through 18 months, coincides with the findings reported here on Diodrast diffusion rate changes in the rat.

The conclusion that this decrease in the Diodrast diffusion rate is due to a change in the synovial ground substance is supported by Gersh and Catchpole's ('49) observation that the dermal ground substance of fetal and newborn rats was easily extracted from frozen and dried sections by a pH 7.0 phosphate buffer solution, but that the ground substance of rats older than 42 days was not extractable. The authors interpreted this as due to a "progressive polymerization of glycoprotein resulting in decreased solubility and extractability" of the ground substance.

The prolonged removal time in old rats indicates a decrease in synovial permeability due to a diminished synovial blood supply. It is not known whether this has a vasomotor or anatomical origin.

Adrenal gland effects on synovial permeability

Seifter et al. ('49) state that synovial permeability in the rabbit is not altered "shortly" after adrenalectomy. Later investigation by Baeder and Seifter ('52) does not clearly in-

dicate whether adrenalectomy has an effect on synovial permeability. Opsahl ('49) found that adrenalectomy in mice was followed by a marked enhancement of intradermal spreading but this effect could only be demonstrated in live animals. She suggests that because the effects of adrenalectomy do not show in dead mice, an unimpaired circulation is necessary. The increase in synovial permeability, however, following adrenalectomy as described in the present paper can be demonstrated in live and dead rats and therefore is not influenced by the animals' circulation.

Seifter ('49) reported a decreased synovial permeability and antagonism of hyaluronidase in rabbits following cortisone injection. Hechter ('50), however, found that cortisone does not inhibit hyaluronidase *in vitro* nor in rabbit skin and thus he doubts that cortisone acts directly on the hyaluronidase mechanism. This effect of cortisone was also questioned by Paul et al. ('52) who observed a marked clinical response to adrenocorticotropic hormone and cortisone in rheumatoid arthritis patients without a corresponding change in the renal excretion of phenolsulfonphthalein after its injection into the knee joint cavity. These investigators were also unable to demonstrate an antihyaluronidase effect of cortisone in rabbits. The insignificant decrease in synovial permeability following the intraarticular injection of cortisone described in the present paper was probably not a real one because the particles of cortisone were too large to leave the joint cavity by diffusing through the synovial tissue. They remained in the joint cavity and acted as a physical hindrance to the passage of Diodrast.

The majority of reports indicates that desoxycorticosterone acetate has no effect on tissue permeability. Edlund ('49) could demonstrate no effect on the absorption of hemoglobin from injured synovial tissues, and Opsahl ('49) could detect no change in the intradermal spreading of India ink in the mouse following treatment with this steroid. Hechter ('50) working with components of ground substance *in vitro* found them unaltered by desoxycorticosterone acetate and obtained

no change *in vivo* as evaluated by intradermal spreading reactions in rabbits. Opposed to these observations is Seifter's ('49) report of a maximal increase in synovial permeability in the rabbit 30 to 90 minutes after the intramuscular injection of this compound. An insignificant increase in the Diodrast diffusion rate following the intraarticular and intraperitoneal injection of desoxycorticosterone acetate was seen in the present work.

The Diodrast diffusion rate measurements indicate that adrenalectomy increases synovial permeability in rats two to 5 months old by acting on the synovial ground substance. The adrenals apparently exert their influence on synovial permeability at some intermediate stage in the synthesis of their hormones because the injection of cortisone and desoxycorticosterone acetate does not have a significant effect on synovial permeability. The metabolism of ascorbic acid, the oxidation products of which are known to be hyaluronidase inhibitors (Patterson and Cole, '52), might be the vehicle for this adrenal activity. Dumm et al. ('52) have reported a large decrease in the excretion of ascorbic acid following hypophysectomy or adrenalectomy in rats.

SUMMARY AND CONCLUSIONS

The influence of certain experimental and natural variables on synovial permeability was measured in rat knee joints using the Diodrast diffusion rate and removal time methods. Testicular hyaluronidase, injected intraarticularly, caused an increase in synovial permeability as shown by an increased Diodrast diffusion rate. Alterations in the synovial blood supply due to blood-letting, physiological shock, or death, all caused decreases in synovial permeability which were indicated by prolonged removal time measurements. From these experiments it was concluded that synovial permeability in the rat can be altered by two means: (1) a change in the synovial ground substance; (2) a change in the synovial blood supply.

The Diodrast diffusion rate method is specific for detecting changes in the synovial ground substance. A decrease in synovial permeability at the approximate age of two months was revealed by this method. This permeability decrease was not prevented by gonadectomy.

The Diodrast removal time method, which is specific for detecting changes in the synovial blood supply, revealed a decrease in synovial permeability in old rats.

Adrenalectomy in rats two to 5 months old caused a marked increase in synovial permeability as demonstrated by the Diodrast diffusion rate method. The intraarticular or intraperitoneal injection of cortisone and desoxycorticosterone acetate did not have a significant effect of synovial permeability.

LITERATURE CITED

BAEDER, D. H., AND J. SEIFTER 1952 The effect of adrenalectomy and hypophysectomy on synovial membrane permeability in rabbits. J. Pharm. and Exp. Therap., *106:* 372.

BENSLEY, S. H. 1950 Histological studies of the reactions of cells and intercellular substances of loose connective tissue to the spreading factor of testicular extracts. Ann. New York Acad. Sc., *52:* 983–988.

BUNTING, H. 1950 The distribution of acid mucopolysaccharides in mammalian tissues as revealed by histochemical methods. Ann. New York Acad. Sc., *52:* 977–982.

DUMM, M. E., E. P. RALLI AND I. GRAEF 1952 Effect of hypophysectomy on pigmentation and ascorbic acid excretion in black rats. Proc. Soc. Exp. Biol. and Med., *79:* 214–216.

DURAN-REYNALS, F. 1942 Tissue permeability and the spreading factors in infection. Bact. Rev., *6:* 197–252.

DURAN-REYNALS, F., H. BUNTING AND G. VAN WAGENEN 1950 Studies on the sex skin of *Macaca mulatta*. Ann. New York Acad. Sc., *52:* 1006–1014.

EDLUND, J. 1949 Studies on absorption of colloids and fluid from rabbit knee joints. Acta Physiol. Scandinav. *18* (suppl. 62): 1–108.

GERSH, I., AND H. R. CATCHPOLE 1949 The organization of ground substance and basement membrane and its significance in tissue injury, disease, and growth. Am. J. Anat., *85:* 457–522.

HECHTER, O. 1950 The ground substance of the mesenchyme and hyaluronidase. Ann. New York Acad. Sc., *52:* 1028–1040.

HIDALGO, J., C. D. MCCLURE, J. B. HENDERSON, R. W. WHITEHEAD AND C. J. SMYTH 1952 Effect on joint permeability of adrenal cortex extract, cortisone, hydroxyphenyl cinchoninic acid and hyaluronidase. Proc. Soc. Exp. Biol. and Med., *80:* 97–101.

JAWORSKI, A. A., J. E. FARLEY, J. BARRETT AND R. JAWORSKI 1950 Relation of hyaluronidase to salicylates and rheumatic fever. J. Ped., *37:* 697–708.

LURIE, M. B., AND P. ZAPPASODI 1939 Relative spread of particulate and diffusible substances in the skin of male and female rabbits. Proc. Soc. Exp. Biol. and Med., *42:* 741–744.

MAUNSELL, K., F. WRIGLEY, T. C. HIGHTON AND L. B. HOLT 1950 The synovial membrane. Some observations in normal and arthritic human joints. Ann. Rheum. Dis., *9:* 363–371.

MEYER, K. 1950 The mucopolysaccharides of the interfibrillar substance of the mesenchyme. Ann. New York Acad. Sc., *53:* 961–963.

OPSAHL, J. C. 1949 The influence of hormones from the adrenal cortex on the dermal spread of india ink with and without hyaluronidase. Yale J. Biol. and Med.,, *21:* 255–262.

PATTERSON, J. W., AND J. W. COLE 1952 A suggested role for vitamin C in connective tissue formation. Anat. Rec., *112:* 372–373.

PAUL, W. D., R. E. HODGES, R. W. KNOUSE AND C. S. WRIGHT 1952 Effect of anti-rheumatic drugs on synovial permeability. Proc. Soc. Exp. Biol. and Med., *79:* 68–71.

SEIFTER, J., D. BAEDER AND A. BEGANY 1949 Influence of hyaluronidase and steroids on permeability of synovial membrane. Proc. Soc. Exp. Biol. and Med., *72:* 277–282.

SEIFTER, J., D. H. BAEDER AND A. BLUMENTHAL 1952 Technical factors influencing the permeability of synovial membrane. Federation Proc., *11:* 389.

A STUDY OF REGENERATES EMANATING FROM LIMB TRANSPLANTS WITH REVERSED PROXIMODISTAL POLARITY IN THE ADULT NEWT

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NINE FIGURES

INTRODUCTION

In attempting to discover the nature of a living system, fruitful results are usually obtained from experiments designed to demonstrate the functioning of that system under a variety of conditions. Numerous experiments of this sort have been conducted in the study of limb regeneration in the amphibia. As has been pointed out by Butler ('51), several investigators have in the past made some examination of regenerates growing from parts of limbs which had been transplanted with their proximodistal axes reversed.[2] However, papers published by these earlier workers have dealt with relatively small numbers of specimens and have cited results which were somewhat varied. In 1949 Butler described a technique by means of which reversed transplants were readily obtained in larvae of *Amblystoma maculatum* and *Amblystoma opacum*. The present investigation is concerned with results produced through the application of that technique to adults of

[1] Work at the Oak Ridge National Laboratory performed under Contract 7405-eng-26 for the Atomic Energy Commission.

[2] It is not strictly correct to refer to this type of abnormal growth as a regenerate since it does not replace a structure which previously existed. However, these growths are apparently produced by a process which differs from typical regeneration only in that the region from which the growth emanates has its proximodistal polarity reversed.

Triturus viridescens viridescens (Rafinesque). In addition, growth of transplants innervated by deviated nerves and of transplants transected at various levels along the proximo-distal axis was observed. Some of the data presented have been cited in a preliminary paper (Dent, '50).

MATERIALS AND METHODS

The adult newts used in this study were collected in Albemarle County, Virginia and were kept at room temperature throughout the investigation. They were maintained on a diet

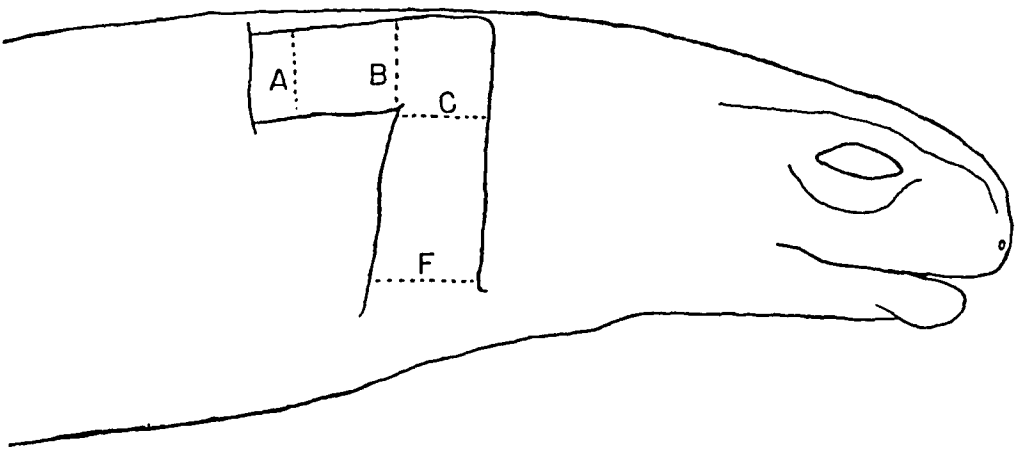

Fig. 1 Diagram showing the position of the limb at the conclusion of step 1 in the transplantation operation described in the text and the 4 levels at which limbs were resected in step 2 of the operation.

of ground lean beef fortified with calcium phosphate (dibasic) and cod-liver oil. Operations were performed under a dissecting microscope on animals anaesthetized by immersion in an aqueous solution (0.2% of tricaine methanesulfonate).

Parts of right forelimbs were transplanted after the method of Butler ('49, '51). This operation was accomplished in two steps. First, the limb was cut through in the midcarpal region, the amputated portion discarded, and the skin removed from the distal portion of the stump proximally to the level of the distal ends of the radius and ulna. A pocket was made in the epaxial trunk musculature approximately 2 mm posterior to

the shoulder region and the skinned portion of the limb stump was inserted into the pocket. This completed step 1. After two weeks the stumps were healed firmly in the pockets and step 2, which involved merely a second transection of the limb at one of four levels, was carried out (see figs. 1 and 7). In this way the limb was in each instance divided into two parts: (a) a transplant, with its proximodistal polarity reversed, attached to the trunk in the pocket and, (b) a limb stump retaining its original polarity and its original attachment to the pectoral girdle. The experimental animals were divided into four groups in each of which the resection was made at a different level. In group A the transection of step 2 was carried out through the radius and ulna near the carpal region. In group B the resection was made through the radius and ulna near their articulation with the humerus; in group C through the humerous near the elbow; and in group F through the humerus near its head. Two subgroups, BD and FD, were also established. The treatment of these animals differed from that of those of groups B and F only in that as a preliminary procedure to transplantation postaxial brachial (flexor) nerves were dissected free from the left forelimbs and from the trunk as far in as the brachial plexus. These nerves were then deviated beneath the skin across the back so that their free ends came to lie in the region where the bottom of the pocket was eventually to be located. After a healing period of two weeks, step 1 followed by step 2 of the transplantation procedure was carried out.

Finally, from groups B and BD there were set aside, respectively, 21 and 13 animals on which the transplantation operation had been completed 100 days previously. All of the transplants from group BD and 7 of those from group B bore well-formed regenerates; the remaining transplants of group B had given no evidence of regeneration. The transplants were then sectioned again just proximal to the level of the second cut so that the regenerates and the tips of the static transplants were removed.

In all, 187 animals were operated upon — 19 in group A; 45 in group B; 38 in group C; 38 in group F; 28 in group BD; and 19 in group FD.

Representative limbs were sectioned at 10 μ, stained with iron hemotoxylin, and counterstained with eosin-azure II.

RESULTS

Patterns of response to operation

Based on original observations and on observations reported by numerous other investigators, Singer ('52) has set forth a generalized description of the pattern of regeneration in limbs of the adult newt. He divides the process into 8 stages. During the period of stage 1, which lasts about two weeks, the limb stump passes through a regulative phase in preparation for the growth of the blastema. Within 12–24 hours (at 25°C.) following amputation the wound surface becomes covered over by a thin sheet of epidermal cells which migrate from the edges of the wound. Beneath this covering there takes place a localized regression of tissue — a characteristic wound reaction. The debris produced by the degenerating cells is then removed by phagocytes and replaced by connective tissue cells. Finally, the regressive activity stops and stage 1 is terminated by the accumulation of blastema cells in this region. Subsequent stages encompass the growth and differentiation of the blastema.

In the present investigation, after the limb was resected in step 2 of the transplantation operation, the stump of the limb retaining its attachment to the pectoral girdle regenerated a right forelimb, following the typical pattern described by Singer. In the transplanted portions of the limb, epidermal cells likewise migrated out over the wound surface at the free end of the transplant during the 24-hour period following resection. The phase of tissue regression and phagocytosis followed. In the transplants of groups A, B, and BD, this phase lasted about two days and degeneration was of about the same degree as that ordinarily observed in typical stumps of am-

putated limbs. In groups C, F, and FD, where resection was through the humerus, degeneration varied in extent from transplant to transplant. In roughly half of these animals, the transplants showed regression of about the same order of magnitude as that seen in typical regenerating limbs, but in approximately 25% the whole of the transplanted portion of the upper arm became decolorized and sloughed away, leaving the tips of the radius and ulna exposed. The remainder of the transplants lost some tissue by sloughing but retained some part of the humerus and its adjacent soft tissues. After the degenerating tissue had sloughed away, the exposed underlying tissues were again covered by epidermis.

Circulation in the transplant was interrupted at the time of resection but was re-established in the second and third days following resection. It reappeared in the vessels nearest the pocket and then spread toward the free end of the transplant.

At the end of the phase of degeneration, apparently, irrespective of the amount of tissue that had been resected in one of two ways. They either persisted as relatively static, nongrowing structures or they produced regenerates which followed the typical pattern of development described by Singer ('52) but which were of left-limb symmetry.

When the transplants failed to develop blastemata, caps of fibrous connective tissue formed at their free ends and the skin covering these caps became reconstituted in the form of that covering the remainder of the transplant (fig. 3). The histological structure of these static transplants was essentially the same as that of the denervated and nonregenerating root limbs described by Rose ('48). Once the phase of active regression was completed, no further resorption or loss of tissue was detected.

Composition of regenerates

The composition of the regenerate varied with the level of the proximodistal axis at which the blastema formed (figs. 2, 7, 8, and 9). From the transplants of group A, which were fitted

carpals and the adjacent distal tips of the radius and ulna there regenerated distal tips of radius and ulna with carpals and digits distal to them (fig. 4). In transplants of groups B and BD the resection of step 2 in the transplantation operation was made through the original proximal ends of the radius and ulna; the blastema formed at this level and the regenerate reproduced the structures which originally lay distal to this level (fig. 5). Similarly, in groups C, F, and FD, the regenerate always reproduced that portion of the transplant which had originally lain distad to the level at which blastema formation took place. In figure 6, for example, parts of the original

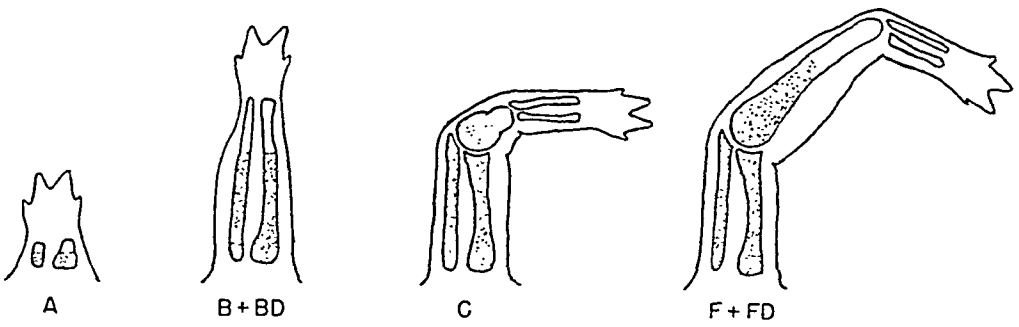

Fig. 2 Diagram representing the extent of the long bones in transplants (and regenerates) in which blastema formation took place very near the level of resection; i.e., in which tissue regression was minimal. The regenerated portions of the long bones are unstippled.

radius, ulna, and humerus can be seen quite clearly. Attached to the original humerus is an equivalent portion of regenerated humerus which articulates with a regenerated radius and a regenerated ulna. This section does not show the most distal portions of the regenerate but they were complete.

The regenerates from the transplants exhibited no structural aberrations when viewed externally, although histological study revealed derangements of the carpals ranging from fusion into a single mass to a condition in which only the first second and the third and fourth distal carpals were fused. Partial fusion of the radius and ulna was also seen. The estimated volumes of the transplant regenerates were usually about two-thirds those of the regenerates from the untrans-

planted stumps among groups A and B, and in groups C and F the disparity in size was usually even greater (figs. 7 and 9). The trend toward fusion of skeletal parts and reduction in size was less marked in transplants supplied with deviated nerve; i.e., groups BD and FD.

Symmetry of regenerates

As was stated earlier, the regenerates which grew from the transplants were left-limb parts, i.e., they possessed a form which was the mirror image of that of the transplants from

TABLE 1

Number of limb parts transplanted in reversed proximodistal polarity in each experimental group and distribution of static and regenerating transplants for the entire series

GROUP	TOTAL NO. OF TRANSPLANTS	NO. OF STATIC TRANSPLANTS	NO. OF REGENERATING TRANSPLANTS	PERCENTAGE OF TRANSPLANTS REGENERATING
A	19	13	6	31.6
B	45	33	12	26.6
BD	28	4	24	85.7
C	38	27	11	28.9
F	38	21	17	44.8
FD	19	6	13	68.4

which they grew. For example, in growing transplants of group B the ulna of the regenerate was continuous with the ulna of the transplant and at either end of the single bone which the two formed there was situated a fused intermedium-ulnare (the fusion of the intermedium and ulnare is typical of *Triturus viridescens*). In a similar relation were situated the radiale of the transplant, the radius of the transplant, the radius of the regenerate, and the radiale of the regenerate. Figure 6 is representative of the spatial relations of the long bones of those growing transplants from groups C, F, and FD in which part of the humerus persisted after the cessation of the phase of regression. It is seen that the regenerated humerus is continuous with the humerus of the transplant and

that the regenerated radius and ulna are on the same sides of the long axis of the limb as are the transplanted radius and ulna. These relations are shown diagrammatically in figure 2.

Frequency of regeneration

In table 1 are shown, for each of the operative groups, the numbers of transplants which remained static and the numbers which produced limb parts. By comparing the percentage of regenerates in group B with the percentage in group BD and the percentage in group F with FD, it can be seen that when transplants were supplied with nerves deviated from left forelimbs the percentage which gave rise to regenerates was more than trebled for the B group and greatly increased in the F group. That the deviated nerves actually innervated the transplants was abundantly clear, since the regenerates of such transplants moved actively and extensively from the time that digit formation was well underway. Also, the stumps of the left forelimbs from which the nerves were deviated retained some innervation and moved in coordination with the limbs regenerated from untransplanted portions of the right forelimbs, whereas the movements of the regenerates on the transplants took place simultaneously and in unison with the movements of the left forelimb stumps. Regenerates from transplants without deviated nerves (those of groups A, B, C, and F) underwent only slight movements resulting, apparently, from contractions of the trunk musculature into which their tips were implanted. There was no flexion or extension of their digits such as was observed in the regenerates of Butler's series ('51).

Regeneration after secondary amputation

Second regenerates formed on all thirteen transplants of group BD from which the original transplants were removed 100 days after operation and on 6 of the 7 transplants of group B which received the same treatment. Regenerates formed on 7 of the 14 static transplants of group B which had had their distal tips removed at the same time.

DISCUSSION

It was discovered at a rather early date (Todd, 1823) that the salamander limb must be innervated in order for it to regenerate. Singer ('46) has demonstrated that regeneration occurs only when a certain threshold number of nerve fibers is present. Since in the present study the percentage of regenerates was very markedly greater among those transplants which received deviated nerves (groups BD and FD) than among similar transplants which did not (groups B and F), it is reasonable to conclude that the failure of the static transplants to produce regenerates can be attributed, primarily, to lack of proper nerve supply. The fact that fibrocellular caps typical of the stumps of the amputated, denervated limbs described by Rose ('48) formed at the tips of nongrowing transplants can be taken as further evidence to support this conclusion. It was observed that when the tips of 14 static transplants of group B were removed, 7 of those transplants produced regenerates. This can be explained by postulating that nerve fibers failed to penetrate to the terminal ends of those static transplants in sufficient numbers to stimulate blastema formation before growth inhibiting cicatrization occurred and that regeneration took place in some instances after the cicatrices were removed because by the time of removal the numbers of fibers which had reached the wound area exceeded the threshold number required for regeneration.

The relation of the reinnervation of the transplant to the halting of the wave of degeneration should also be considered. Denervated larval limbs regress completely after distal injury unless reinnervation takes place, in which instances the wave of regression is rapidly halted (Butler and Schotté, '49; Thornton and Kraemer, '51). In Butler's study ('51) of inverted larval limb parts the transplanted portion of the upper arm regressed completely or almost completely. In the present study the impression was gained that there was an inverse correlation between the rapidity with which circulation was re-established in the transplants and the amount of tissue lost

by sloughing. The precise observations necessary to confirm this impression would have been extremely difficult to make. However, following the operation, blood did begin to circulate first in the vessels nearest the base (pocketed end) of the transplant, and less regression of tissues occurred in the shorter transplants where resection was made through the forearm than in the longer transplants which included portions of the upper arm. The re-establishing of circulation may be as important as reinnervation in the halting of the degeneration of the transplant, particularly in the adult newt where denervated limbs do not ordinarily regress completely after terminal injury (Rose, '48).

Butler ('51) reported that none of the transplants made by him regenerated upper arm parts. In fact, when residual pieces of humerus remained after the phase of regression, they appeared to inhibit regeneration. As has been pointed out numerous transplants of groups C, F, and FD produced regenerates which included segments of the upper arm. Possibly the factors involved in the difference in reaction to amputation after denervation are also involved in this difference in regenerative response between *Amblystoma* larvae and adult newts.

It is well known that regeneration in normal polarity can occur at any level on the long axis of the limb and that the regenerate reproduces, essentially, neither more nor less than the part removed. Butler ('51) raises the question of whether or not the local character of blastema formation is such that any level of the limb possesses the capacity for producing a blastema in a proximal as well as in a distal direction. The results presented here show that segments of limb with the proximodistal axis reversed are capable of giving rise to regenerates at any one of 4 levels, quite probably any level, on that axis and that the extent of the regenerate varies with the level at which regeneration begins. If a normal limb is amputated near the head of the humerus the regenerate is composed of the structures which would ordinarily lie distad to that region. Regeneration at that level from tissues with reversed polarity (as in

the growing transplants of groups F and FD) produces the same complement of limb. If, at the other extreme, a normal limb is cut through just above the wrist, a new hand and wrist are regenerated. A similar production of structure from the same level was seen in the reversed transplants of group A. This principle is also borne out in the engrafted limb parts of groups B and C.

The symmetry of regenerates emanating from reversed transplants, as was observed by Milojevic and Grbic ('25), is the mirror image of the symmetry of the transplant. It will be recalled that the transplants of the present investigation were all pieces of right limbs. The regenerates were all left-hand parts. This is readily interpreted to mean that, although the proximodistal axis is reversed, the anteroposterior and dorsoventral axes remain unchanged and that, whereas the first mentioned axis is labile, the other two are apparently fixed. The limb field is plastic enough to compensate for the drastic change resulting from the reversal of one of the three major axes and to produce an integrated and essentially complete limb.

SUMMARY AND CONCLUSIONS

1. Utilizing a technique of Butler ('49, '51), parts of right forelimbs from adult specimens of *Triturus viridescens viridescens* (Rafinesque) were transplanted in reversed proximodistal polarity.

2. Regenerates formed on many of these transplants, but the others persisted as static, nongrowing members.

3. The percentage of transplants which formed regenerates was increased greatly when nerves from left forelimbs were deviated to the sites of transplantation.

4. The limb in reversed proximodistal polarity, like the typical limb, has the capacity for blastema formation at any level on its long axis.

5. The regenerate from the reversed transplant reproduces in mirror image those structures normally distal to the level of blastema formation.

LITERATURE CITED

BUTLER, E. G. 1949 Reversal of polarity in limbs of urodele larvae. Biol. Bull., *97:* 232–233.

———— 1951 The mechanics of blastema formation and regeneration in urodele limbs of reversed polarity. Trans. New York Acad. Sc., *13:* 164–167.

BUTLER, E. G., AND O. E. SCHOTTÉ 1949 Effects of delayed denervation on regenerative activity in limbs of urodele larvae. J. Exp. Zool., *112:* 361–392.

DENT, J. N. 1950 Reversal of proximo-distal polarity in limbs of adult newts. Anat. Rec., *108:* 522.

MILOJEVIC, B. D., AND NATALIJA GRBIC 1925 La régénération et l'inversion de la polarité des extrémités chez les Tritons adultes, a la suite d'une transplantation hétérotope. Compt. Rend. Soc. Biol., *93:* 649–651.

ROSE, S. M. 1948 The role of nerves in amphibian limb regeneration. Ann. New York Acad. Sc., *49:* 818–833.

SINGER, M. 1946 The nervous system and regeneration of the forelimb of adult Triturus. V. The influence of number of nerve fibers, including a quantitative study of limb innervation. J. Exp. Zool., *101:* 299–338.

———— 1952 The influence of the nerve in regeneration of the amphibian extremity. Quart. Rev. Biol., *27:* 169–200.

THORNTON, C. S., AND D. W. KRAEMER 1951 The effect of injury on denervated unamputated limbs of Amblystoma larvae. J. Exp. Zool., *117:* 415–440.

TODD, T. J. 1823 On the process of reproduction of the members of the aquatic salamander. Quart. J. Lit., Sc. and Arts, *16:* 84–96.

PLATES

PLATE 1

EXPLANATION OF FIGURES

3 Longitudinal section cut through the free end of a nongrowing transplant from group C. The bone in the central region of the photograph is a residual portion of the humerus which is covered over by a fibrous cap typical of nonregenerating limb stumps.

4 Longitudinal section from a regenerating transplant of group A. Considerable fusion of skeletal elements is evident but the darkly stained transplanted portions of radius and ulna can be distinguished from the more lightly stained bones of the regenerate.

5 Longitudinal section from a regenerating transplant of group B. The residual radius and ulna are seen to be continuous with the regenerating radius and ulna. Parts of three carpals are shown but the other carpals and phalanges of this limb are not present in this section.

6 Longitudinal section from a transplant of group F. Here parts of the transplanted radius and ulna are visible on the left. They articulate with a transplanted portion of humerus from which extends a more lightly stained portion of regenerated humerus. The regenerated humerus, in turn, articulates with a regenerated radius and a regenerated ulna on the right. The distal portion of the regenerate does not appear in this section but was typical in form.


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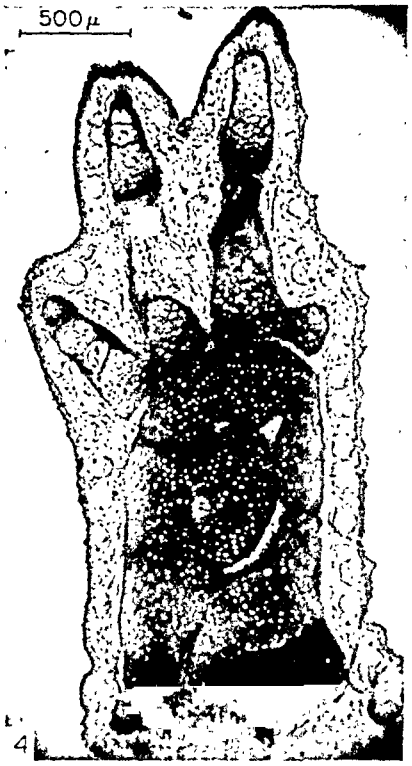
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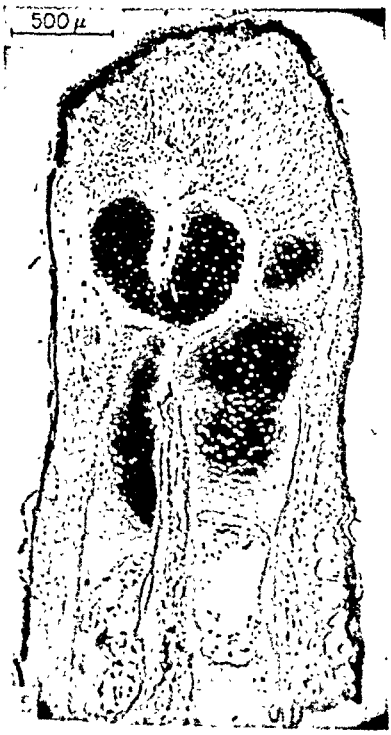
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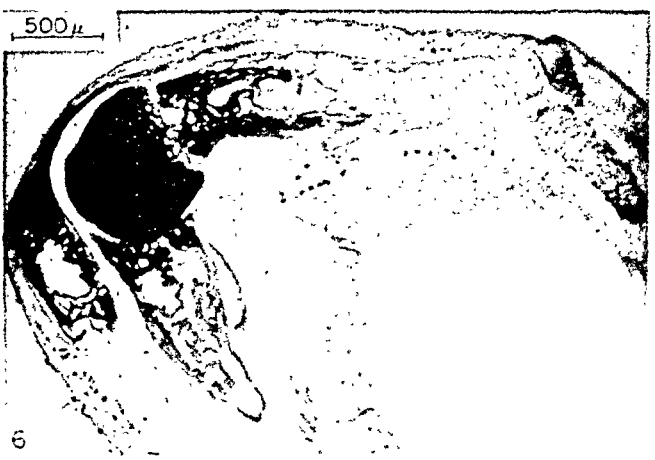
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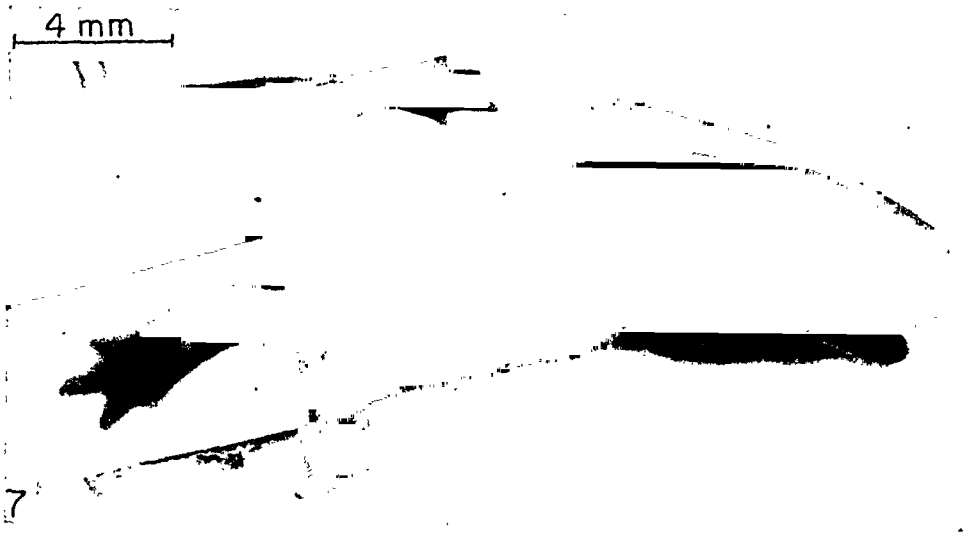

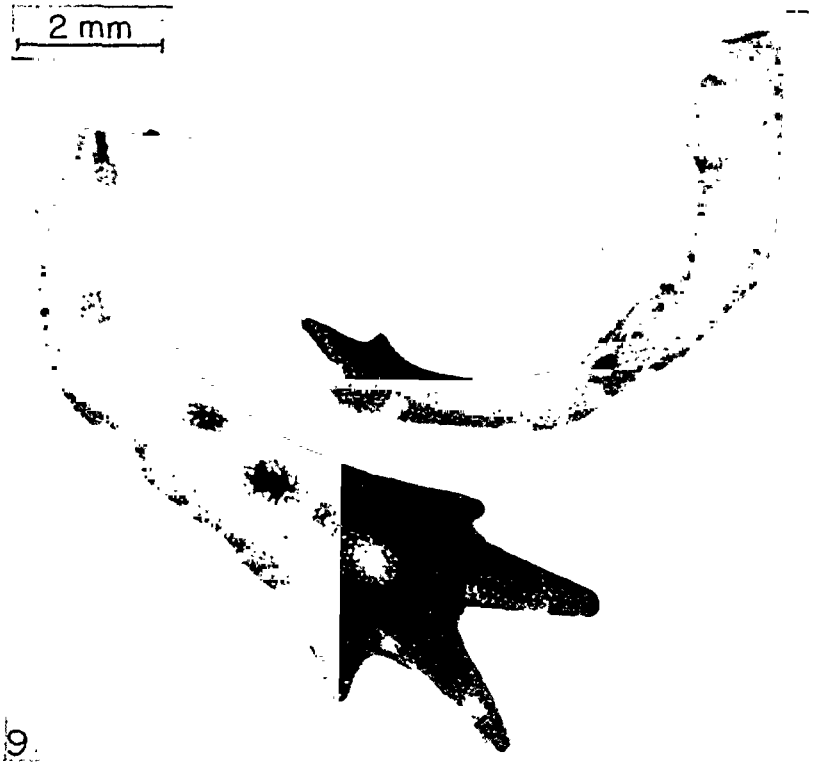

7 Preserved specimen from the BD group showing regenerates from the transplanted and untransplanted portions of the right forelimb.

8 Regenerating transplants from group A (left) and group FD (right). Preserved material.

9 Transplanted (left) and untransplanted (right) portions of a preserved limb from group F. Note the difference in size between the two regenerates.

CONTROL OF HYPERGLYCEMIA OF DIABETIC RATS BY PARABIOSIS WITH NORMAL RATS [1]

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SIX FIGURES

Functional and morphologic changes occur in the animal organism whenever adaptations to altered internal or external environment occur. In the presence of antithyroid drugs the thyroid gland, for example, hypertrophies to compensate for lowered levels of thyroxin. Changes in the histochemistry of the pituitary body arise in response to demands for increased hormonal production. Hyperplasia of one of a pair of organs ensues upon the loss of the other.

Such compensatory changes in an animal are largely obviated when it is joined organically to another as in parabiosis, providing thereby added sources of active substances which may be delivered through a common circulatory system to the member of the pair deficient in them. Thus parabiosis has proved a useful tool in studying the influences exerted upon one parabiont by modifying the internal environment of the other. So extensively has the technic appealed to investigators that Finerty ('52), in his recent

[1] Work represented in this paper was carried out in the Section of Anatomy of the Mayo Clinic and Mayo Foundation.

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excellent review article, listed 14 physiologic areas in which the procedure of parabiosis has proved useful for study.

According to Finerty ('52), Bert in 1862 was the first investigator to join albino rats by parabiosis and to demonstrate a cross circulation between them. Sauerbruch and Heyde ('08 and '09) joined rabbits in this manner, studying the toxicity incited in one by developing ileus in the other. Then Forschbach ('08), using dogs, pancreatectomized one and observed the severity of diabetes as modified by the normal pancreas in the other. Joining rats, Mayeda ('21) observed the maintenance of a totally adrenalectomized parabiont by the hyperfunctioning adrenal glands of the other. A nephrectomized animal was shown to live for weeks sustained entirely by the functional kidney of its mate (Herrmannsdorfer, '23). But the weights of the functional kidney exceeded those of a normal by three times (Jeffers et al., '40). Later it was shown that the hypertension of the nephrectomized parabiont was not prevented by the hyperfunctioning normal parabiont, indicating that such surgically joined individuals retained independent circulatory pressure adjustments (Grollman and Rule, '43).

There has been considerable evolution in the parabiotic technics employed over the years. The early procedures joined only skin flaps. Later, the deeper muscles were included; but Hill ('32) cut the abdominal musculature, and then sutured the 4 cut surfaces, forming thereby a more suitable bridge for capillarial extension. To restrict movement further, however, and thus to lessen trauma, Bunster and Meyer ('33) joined the lateral aspects of the scapulae of the two parabionts.

A number of studies have demonstrated capillarial continuity between the parabionts, but Hill ('32) showed that at least 6 hours were required to obtain an equal distribution of brilliant vital-red dye to each of the partners when it was injected into the vascular bed of one. Various materials have been employed to study the extent of cross circulation. Iodine, immune bodies, bacteria, particulate substances, and

foreign erythrocytes have been used. A more recent technic employed erythrocytes tagged with radioactive iron (Van Dyke et al., '48). Data assembled by these authors showed that approximately 0.64% of the total red-cell mass flows from one animal to the other per minute, and that transfer was detectable on the second postoperative day.

Our interest in the study of experimental diabetes, employing parabiosis, grew out of an observation of one of us (Weitze, '45) that the pituitary glands of normal mice failed to provide sufficient growth hormone to sustain satisfactory growth in their hypophysectomized parabionts. Sometime before, Møller-Christensen ('33) had stated that in some instances sufficient amounts of growth hormone had been produced by a normal parabiont to promote minimal growth in its hypophysectomized mate. Thus this observation on parabiosed mice prompted our study of the capacity of the normal pancreas of rats to maintain normal sugar levels in its hyperglycemic parabiotic mate, which had been made diabetic either by pancreatectomy or by giving alloxan which is known to destroy the islet cells of the pancreas.

While our study was in progress the report of Shipley and Meyer ('47) who studied alloxan-treated parabionts appeared, and subsequently the work of Fels and Foglia ('49) who had induced hyperglycemia by pancreatectomy was published. Our observations sustained those of both Shipley and Meyer ('47) and of Fels and Foglia ('49) that insulin may be transported through the capillarial bed across the barrier from one parabiont to another in sufficient amounts to restore and to maintain normoglycemic levels in both members of the pair, one of which has been made hyperglycemic by surgical procedure or by chemical toxicity.

MATERIALS AND METHODS

Healthy adult male rats of the Sprague-Dawley strain weighing 160 to 180 gm were used in this study. They were free of respiratory infections or any other obvious disease. Under ether anesthesia litter mates were united according to

the method described by Bunster and Meyer ('33). Pancreatectomy was performed according to a modification of the method of Ingle and Griffith ('49). Alloxan shown by numerous investigators to destroy the sources of insulin in the pancreas was administered subcutaneously in a 5% solution in amounts equal to 150 mg per kilogram of body weight. In some instances alloxan was given one member of a pair after they had been joined, and in other instances it was given to one rat for a considerable period before it was surgically joined to its selected litter mate. Venous blood was taken without a previous fast and blood sugar was determined by the method of Hagedorn ('46). All determinations were made in duplicate.

OBSERVATIONS

Diabetes induced by alloxan. The data obtained from a pair of animals which had been joined by parabiosis prior to the induction of a diabetic state in one of the two are shown in figure 1. In this pair, alloxan was given on the day following parabiotic union, and on the second day after the drug had been given the blood-sugar level was 265 mg per 100 cm^3 in the diabetic animal, whereas it was slightly in excess of 100 mg in its uninjected mate. Determinations made on the 10th postoperative day, or 9 days after alloxan had been given to one of the pair, showed that the blood-sugar levels were essentially alike. Three sets of determinations were made during the ensuing 15 days, during which time but slight differences were observed in the blood-sugar values for this parabiotic pair. Then on the 25th postoperative day, the pair was surgically and aseptically separated, and samples of blood obtained 24 hours later showed that within that brief period a marked disparity in the concentration of sugar had already developed. The level of blood sugar continued to increase in the alloxan-treated animal, whereas in the normal parabiont it decreased immediately but rose slightly during the ensuing 15 days. At the conclusion of

the experiment 20 days after the parabiotic union had been severed, a mean sugar level of 290 mg per 100 cm^3 was again recorded for the diabetic member and a level of 162 mg was recorded for the normal member of the pair.

Data obtained from an experiment wherein alloxan was given an animal 12 days before it was joined by parabiosis to its normal mate are shown graphically in figure 2. When

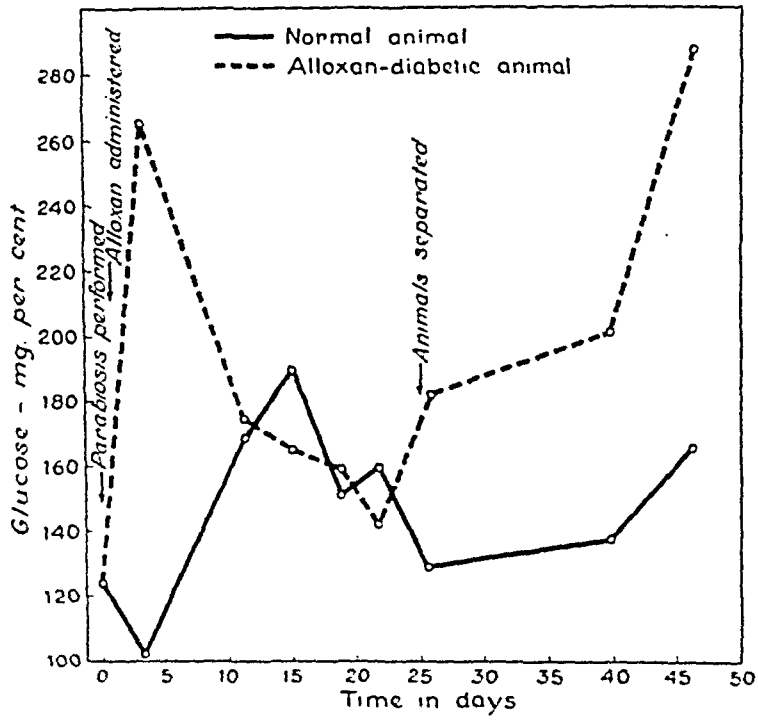

Fig. 1 Levels of glucose in the blood streams of a diabetic and a normal rat during parabiotic union and after separation.

they were joined together, the average blood-sugar level of the diabetic animal was 188 mg per 100 cm^3, while that of the normal animal was 121 mg. In this pair an immediate decline in the blood-sugar level did not occur, for determinations made on the 7th day after parabiosis showed that in the diabetic parabiont a level of more than 200 mg had developed and a corresponding increase was found in the normal mate. Up to this time it would appear, at least in this pair, that the output of insulin was inadequate to restore

or maintain normal glycemic levels. But three days later the levels of glucose were essentially alike, that of the diabetic parabiont being somewhat higher than that of the normal. Five days later the determinations were reversed in that the level of the normal parabiont was higher than that in its diabetic mate.

It is interesting to record that during the interval from the 25th to the 40th postoperative day the surgical union of

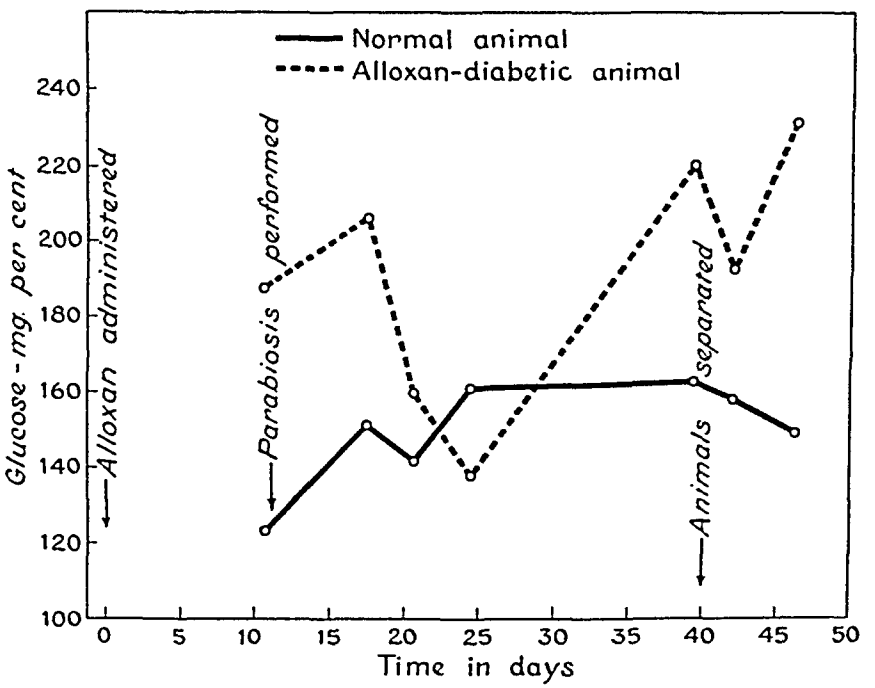

Fig. 2 Levels of glucose when alloxan was given sometime before parabiosis was performed, and the levels after such animals were separated.

these animals became impaired, resulting in a minimal skin connection between them and in the reduction thereby of the capillarial connections. As a result the sugar level in the diabetic member on the 40th day was again high, while the level in its normal parabiont remained unchanged. When these animals were surgically separated on the 40th day after the administration of alloxan, the disparity in their blood-sugar levels again became even greater than that recorded before they were united.

In one set of experiments, the data of which are portrayed in figure 3, parabiosis with a normal rat did not result in restoring the sugar level in the diabetic parabiont to normal as shown in other experiments (figs. 1 and 2). Alloxan was given to one of the pair on the day following their joining. Successive determinations of the sugar made during the ensuing 45 days showed that there had been no effect exerted upon these levels in the diabetic parabiont, by the

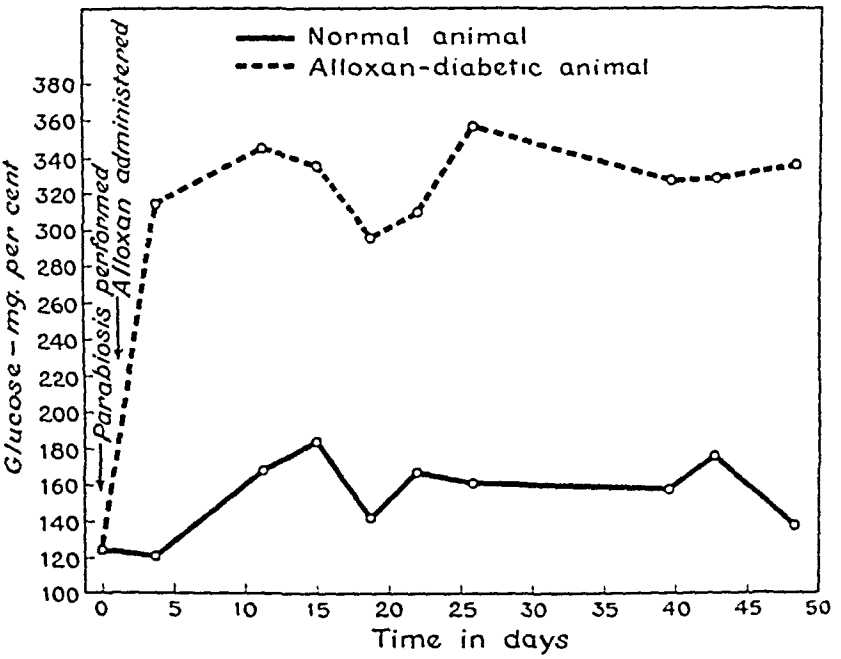

Fig. 3 Levels of glucose in a pair of rats wherein parabiosis did not provide a satisfactory means for the transport of insulin sufficient to lower the hyperglycemia of the alloxan-treated parabiont.

organic continuity with the normal parabiont. During this period of observation, the sugar level in the normal continued to fluctuate within the normal range. There is no ready explanation for this departure from the expected course in this pair. Injections of trypan blue into one parabiont readily entered its mate showing that anastomosing arterial channels were available for the transfer of added insulin across the barrier were the insulin available for transport.

Diabetes induced by partial pancreatectomy. The data obtained upon joining a normal rat to a partially pancreatectomized rat are condensed and shown graphically in figure 4. Blood-sugar levels were essentially identical in the animals at the time they were joined. Seventeen days later, additional pancreatic tissue was removed from the deficient parabiont, but even so, the blood-sugar levels remained essentially alike until the animals were surgically separated

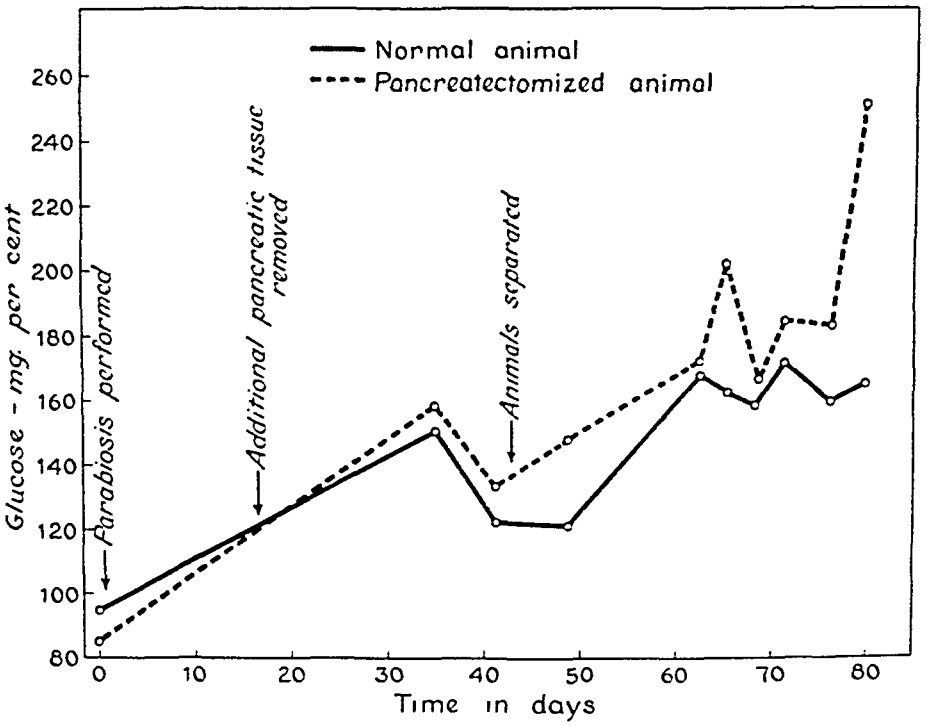

Fig. 4 Levels of glucose in a parabiotic pair of rats, in one of which partial pancreatectomy had been performed. The time of separation is indicated.

41 days after the original parabiosis had been performed. Data were obtained at intervals following their separation but it was not until the 39th day after separation that a wide disparity in the sugar levels between these animals was recorded. The level at that time in the pancreatectomized parabiont was 250 mg per 100 cm^3, while that in the normal parabiont was 160 mg.

When partial pancreatectomy was performed in one rat some time before it was united in parabiosis to a normal rat,

the data differed considerably from those shown in figure 4. Fifteen days after partial pancreatectomy the recorded sugar level was 179 mg per 100 cm^3 (fig. 5). This animal was then joined in the usual way to its unoperated normal mate whose blood-sugar level was then 121 mg. Nine days later, when determinations of sugar were made again, the levels in the two parabionts were essentially alike, being 136 and 141 mg, respectively. Although the blood-sugar levels for both

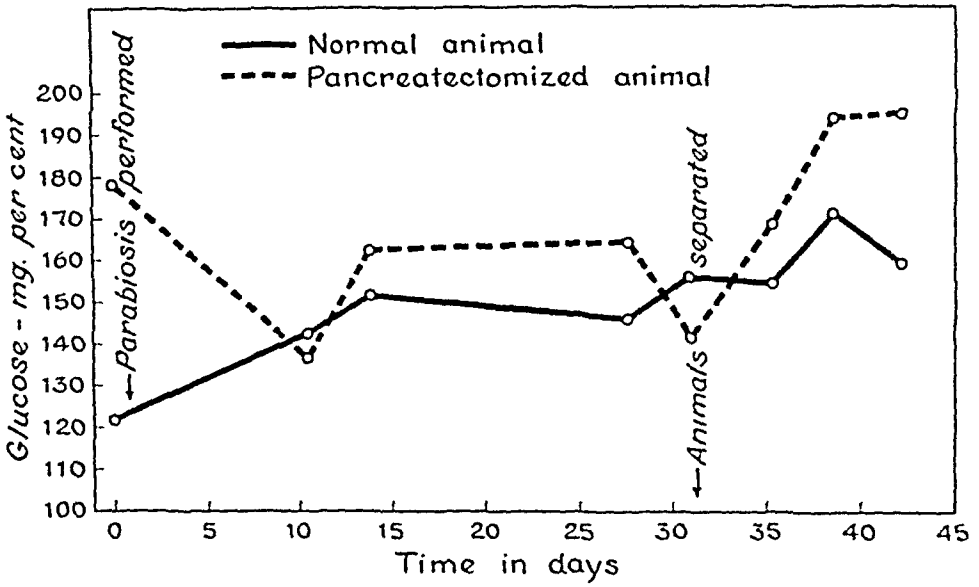

Fig. 5 Levels of glucose in a pair of animals, one of which had been pancreatectomized sometime before parabiosis was done. The time of separation is indicated.

parabionts were higher at subsequent determinations than at the initial sampling following their union, they were not widely divergent from each other during the ensuing period. But following their separation on the 30th postunion day, the sugar levels then became more divergent and at the termination of the experiment on the 43rd day, the high sugar level in the diabetic parabiont recorded before its union with its normal mate was re-established.

The last experiment reported (fig. 6) portrays the data obtained when a normal rat was united in parabiosis to a

diabetic rat which had been partially pancreatectomized 20 days before. Three days before parabiosis was established, the blood-sugar level in the diabetic member was 182 mg, while that of the normal mate to which it would be joined was 122 mg. At the next sampling 4 days after parabiosis, the sugar levels of the two members of the pair were essentially alike.

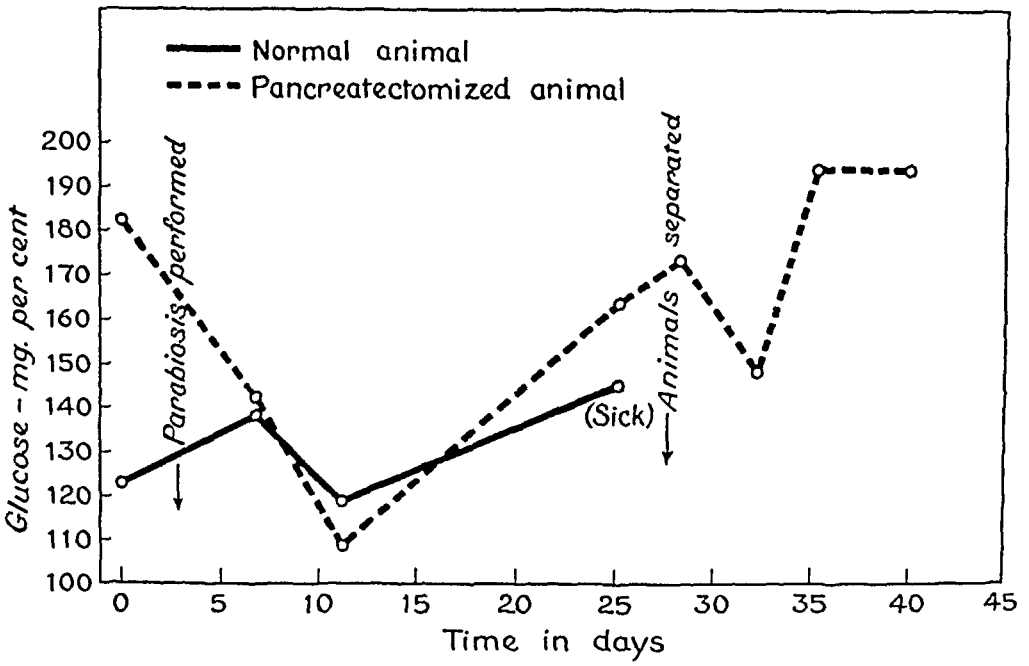

Fig. 6 Levels of glucose in a pair of animals, the normal of which, because of illness, proved inadequate to prevent the hyperglycemia of its pancreatectomized mate.

At subsequent determinations, made 12 and 25 days after parabiosis had been performed, the sugar levels of the two parabionts closely approximated one another. Unfortunately the normal parabiont of this pair developed a respiratory pathologic condition about the 25th day, so that it was freed surgically from its diabetic parabiont at this time. Subsequent determinations on the diabetic survivor showed increased concentrations of sugar, and at the end of the experiment, 12 days after separation, the sugar level in the freed diabetic parabiont was 196 mg, a level higher than recorded before it was joined to its normal mate.

COMMENT AND SUMMARY

A study is reported of the capacity of the pancreas of a normal rat to so extend its function of insulin secretion as to enable it to reduce hyperglycemia in a litter mate joined to itself by parabiosis.

Hyperglycemia was induced either by the intravenous administration of alloxan or by the surgical removal of portions of the pancreas. Parabiosis was performed, using litter mates, either before or after the induction of the hyperglycemic state whether by giving alloxan or by partial removal of the gland.

If alloxan was given to one parabiont, soon after parabiosis, but before the vascular beds became confluent, then a high level of glucose in the injected animal was briefly maintained. As soon, however, as vascular continuity was established, the high level of the hyperglycemic animal dropped to one essentially comparable to that of the normal parabiont which was now considerably higher than it had been before the union of their vascular beds had taken place. The demand made upon the normal pancreas for insulin was obviously greater than it could immediately supply, with the result that although the high levels of the hyperglycemic animal were reduced, the low levels of the normal parabiont were correspondingly increased. But within a week or 10 days, it seemed that larger amounts of insulin were elaborated, thereby lowering the levels of glucose in both the hyperglycemic and the normal parabionts. Then upon surgical separation hyperglycemia was restored in the alloxan-treated animal and normoglycemia maintained in its uninjected mate. Thus we did not encounter hypoglycemia in the severed normal mate, although it must have had a hyperactive pancreas as a result of the dual demand made upon it for insulin during its parabiotic union with its hyperglycemic mate. Shipley and Meyer ('47) likewise did not observe hypoglycemia in their normal animals after separation from their hyperglycemic mates.

When hyperglycemia was well induced by alloxan sometime before the animal was joined to its normal mate, an interval of 8 to 10 days elapsed before the levels of glucose of the two parabionts were essentially alike. And here too, although the level of glucose in the alloxan-treated animal was greatly reduced by the insulin transported across the connecting vascular bed, the level of glucose in the normal mate was considerably increased during that interval.

Shipley and Meyer ('47) had observed that in a certain percentage of their cases reduction of blood glucose did not occur following anastomosis of the blood vessels of the two parabionts. Our data too included a few pairs of parabionts, illustrated in figure 3, in which the hyperglycemia of the alloxan-treated rat was not alleviated by the presence of the normal rat. Vascular continuity between the parabionts was established and yet the effectiveness of insulin transport was not demonstrated.

CONCLUSIONS

In most instances in which parabiosis has been satisfactorily established, resulting in an anastomosis between the vascular beds of two parabionts, the one diabetic and the other normal, the pancreas of the normal mate elaborates increased amounts of insulin, sufficient to maintain normoglycemic levels in both animals. The wide separation of the glucose levels in the blood streams which follows soon after the surgical separation of such animals is proof of the dependence of one animal upon the increased functional activity of the pancreas in the other for the maintenance of normal blood-glucose levels.

LITERATURE CITED

BUNSTER, EDUARDO, AND R. K. MEYER 1933 An improved method of parabiosis. Anat. Rec., *57:* 339–343.

FELS, E., AND V. G. FOGLIA 1949 Diabetes pancreatica y parabiosis. Soc. Argen. de biol., *25:* 62–66.

FINERTY, J. C. 1952 Parabiosis in physiological studies. Physiol. Rev., *32:* 277–302.

FORSCHBACH, J. 1908 Parabiose und Pankreasdiabetes. Deutsche med. Wchnschr., *34:* 910–911.

GROLLMAN, ARTHUR, AND COLTER RULE 1943 Experimentally induced hypertension in parabiotic rats. Am. J. Physiol., *138:* 587–592.

HAGEDORN, H. C. 1946 The absorption of protamine insulin. Copenhagen Steno. Mem. Hosp. Rep. and Nord. Insulinlab., *1:* 25–28.

HERRMANNSDORFER, ADOLF 1923 Experimentelle Nierenstudien an parabiose- und Einzelratten. Ein Beitrag zur Pathogenese der Urämie und Eklampsie. Deutsche Ztschr. f. Chir., *178:* 289–392.

HILL, R. T. 1932 Blood exchange and hormonic reactions in parabiotic rats. J. Exp. Zool., *63:* 203–234.

INGLE, D. J., AND J. G. GRIFFITH 1949 The Rat in Laboratory Investigation. Second edition. J. B. Lippincott Co., Philadelphia. 446 pp.

JEFFERS, W. A., M. A. LINDAUER, P. H. TWADDLE AND C. C. WOLFERTH 1940 Experimental hypertension in nephrectomized parabiotic rats. Am. J. Med. Sci., *199:* 815–818.

MAYEDA, T. 1921 Parabiosis of animals. Deutsche Ztschr. f. Chir., *167:* 295–348.

MÓLLER-CHRISTENSEN, E. 1933 Über einige Parabioseversuche mit hypophysektomierten Ratten. Acta path. et microbiol. Scandinav., *10:* 296–320.

SAUERBRUCH, F., AND M. HEYDE 1908 Ueber Parabiose künstlich vereinigter Warmblüter. München med. Wchnschr., *55:* 153–156.

———— 1909 Weitere Mittheilungen über die Parabiose bei Warmblütern mit Versuchen über Ileus und Urämie. Ztschr. f. Exper. Path. u. Therap., *6:* 33–74.

SHIPLEY, ELVA G., AND R. K. MEYER 1947 Diabetes in parabiotic rats. Am. J. Physiol., *148:* 185–192.

VAN DYKE, D. C., R. L. HUFF AND H. M. EVANS 1948 The efficiency of the vascular union in parabiosis. Stanford M. Bull., *6:* 271–275.

WEITZE, MARIE 1945 The action of the pituitary gland on the growth of mice shown by parabiosis. Acta path. et microbiol. Scandinav., *22:* 151–158.

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