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With the commencement of the present volume (April) of *The Anatomical Record* a change has been made in the editorial management. At the recent meeting of the American Association of Anatomists a committee was elected to appoint editors for the two journals of the society, and to serve as an advisory committee for those so appointed. In the case of *The Anatomical Record* the choice fell on me, and it was decided that I should assume the duties of editor at once. Although given the privilege of selecting one or more associate editors, I have decided not to do so for the present, at least, trusting that fellow members of the society will be willing occasionally to give me the benefit of their opinions of certain articles quite informally.

The policy of the journal will not be materially changed, but an attempt will be made to hasten the publication of accepted articles, to adhere a little more closely to the original plan of *The Record*, and to differentiate it more clearly from THE AMERICAN JOURNAL OF ANATOMY.

In order to accomplish these results, it will be necessary to limit the length of the articles printed. No definite rules can be laid down, but in the opinion of the advisory committee papers of five or ten printed pages will usually be much more acceptable than those necessitating greater elaboration; while notes on laboratory methods, preliminary reports, etc., will be considered appropriate subject matter. All contributors are earnestly and confidently requested to cooperate in this policy.

At this time there are many articles already accepted by the former editorial board of *The Record* and ready for publication. These will, of course, be given precedence over later contributions. I have no hesitation in assuming their value, appreciating as I do, and as, I am sure, do all the readers of *The Record*, the careful and efficient work and the scientific discrimination of the former managing editor and his associates.

JOHN LEWIS BREMER.

All contributions and correspondence should be sent to

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Resumen por el autor, C. B. Moore,
Leland Stanford Junior University.

Infecciones de la uretra femenina.

A causa de su estructura, posición y mecanismo de desplazamiento, la uretra femenina es muy propensa a la invasión bacteriana. En ella se han encontrado una gran variedad de organismos. El gonococcus es el más importante, por su tendencia hacia la cronicidad y sus efectos nocivos. En las glándulas para-uretrales de Skene pueden presentarse infecciones supurativas crónicas, y también en las estructuras vestigiales de la glándula prostática, las cuales emiten pequeñas cantidades de pus de modo indefinido en la uretra anterior, sin que exista ningún síntoma local, por cuya causa no se descubren frecuentemente. La destrucción completa de estas glándulas, tal como puede conseguirse mediante el electro-cauterio, parece ser el único tratamiento que puede terminar de modo permanente las afecciones de dichas partes.

Translation by José F. Nonidez
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INFECTIONS IN THE FEMALE URETHRA

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TEN FIGURES

In order to understand better the infections which occur in the female urethra and especially their tendency often to chronicity and the frequency with which they escape detection, a clinical and laboratory study of female urethras has been made during the last three years, together with a review of some of the literature which might be of assistance. The work in the laboratory comprises some bacteriological investigations and histological examinations of urethras of the newborn, infants, and adults of different ages. For the clinical studies, which were done at the same time in the Women's Clinic of the Stanford University School of Medicine, a new instrument was devised which has proved very satisfactory for the purpose of examining and treating the anterior urethra. Photographs of the microscopic sections and the instrument are appended below.

Because of its structure and location, the female urethra is very prone to bacterial invasion and retention, and for this reason is of distinct clinical interest both to the obstetrician and to the gynecologist. Infections occur here to a great extent only during the child-bearing period and it is not an uncommon focus of infection during pregnancy.

The female urethra is a fibromuscular structure about 35 mm. in length, lying dorsally to the symphysis pubis and ventrally to the distal end of the anterior vaginal wall with which it is intimately associated. It is composed mostly of involuntary muscle fibers interwoven loosely with white fibrous and elastic tissue carrying numerous nerves and blood-vessels in its meshes, the corpus spongiosum. External to this appears two definite

muscular coats, the inner, or longitudinal, and the outer, or circular coat. The latter forms a large ring, the unstriped sphincter, in the region of the vesicular neck; its reinforcement with striped muscle makes the striped or voluntary sphincter. The mucosa lining the canal is stratified squamous epithelium to a varying extent in the anterior end, stratified columnar epithelium in the intermediate region, and a transitional variety in the posterior end near the bladder. At the meatus of some subjects, especially nulliparous ones, the mucosa is extended into two lateral folds, called by Kelly (1) the labia urethrae. They vary in size and shape and undoubtedly assist in protecting the urethra from bacterial invasion from without. The urethral meatus usually lies about 10 mm. from the anterior vaginal wall. In some subjects it lies even closer than this, sometimes a few millimeters. In the mechanism described below the meatus may come even to lie in a plane with the anterior vaginal wall.

The most important feature of the mucosa and the one giving rise to most, if not all, of the pathology is the glands, the most important ones of which are those of Skene. There are also many mucous or sinus glands found throughout its course. The latter structures are lined with short columnar epithelium and are sometimes called Littre glands. Skene's glands, usually two in number, lie beneath the mucosa posteriorly and near the meatus. They are individual, anatomic structures different from the crypts or sinus glands. Doctor Skene (2), of Brooklyn, in 1880, was the first one to discover these structures and realize their importance and to investigate their anatomy and some of their pathology. Dr. J. Kocks (3), of Bonn, and Prof. Max Schüller (4), a couple of years later, also investigated and described these para-urethral glands. The former considered them vestigial structures of the wolffian tubules.

These glands have their genesis in the prostate which is present in both sexes (5). The first anlage of the prostate in the female appears in embryos of 50 mm.; in the male of 55 mm. At first the prostate consists of solid epithelial buds which extend into the surrounding mesenchyme from the epithelium of the urogenital sinus, an early anterior division of the cloaca. Part of

the anterior division of the cloaca goes to make the bladder and the urethra. These buds are most numerous on the dorsal surface, less so on the sides, and rarely on the ventral surface, although they may at times appear around the whole periphery. Those on the dorsal surface develop and branch, while those on the ventral surface remain simple and mostly degenerate. In the male these buds become enveloped in a fibromuscular mass to make the prostate gland. Some of the ducts which were not included in the formation of the prostate gland form accessory glands. According to Keibel and Mall, in the female embryo few glands are formed, three being the maximum number. These may undergo development and form the above-mentioned Skene's glands or ducts. This condition may possibly explain the different degrees of urethral infections in the female, at least to some extent. Since the glands are under retrogressive influences, they may not appear in some cases; in others they may be simple and shallow, in which case the infection is near the surface and therefore more easily cured; in others they may attain greater development, in which case infections become deep-seated and with poor drainage. This latter type leads to the chronic case described below which has been so difficult to cure.

In the embryo there are still other glands which develop in this region. These are the small sinus glands. They appear around the entire periphery of the urogenital sinus in the embryo of 60 mm. These glands have the character of those of Bartholin, but do not attain to the same development. The gland of Bartholin, also developed from the urogenital sinus, comes to lie outside of the urethra, for which reason it does not enter into the present topic. Thus it is revealed from embryonic studies that there is considerable glandular development in this region. According to the distribution of these glands, the urethra is sometimes divided into an anterior and a posterior urethra, the former being the glandular region and the latter the non-glandular region (6).

The structure of Skene's para-urethral glands is described as follows (7): Upon each side, near the floor of the urethra, are

two tubules extending from the vicinity of the meatus for 7 mm. to 15 mm. beneath the mucosa. The mouths of these tubes open upon the surface usually on either side of the median line about 3 mm. from the outer border of the meatus. The upper ends of the tubules terminate in a number of divisions which branch off into the muscular walls of the urethra. These racemose structures are lined by a compound epithelium composed of three layers. The deepest layer is composed of young roundish cells having large, granular nuclei which make up the major portion of the cells. Next above this is a layer of cells of a somewhat spindle shape with prominent nuclei. They are young cells at a more advanced stage than those of the first layer. The next, or outermost, layer is composed of fully developed columnar epithelium with distinct nuclei arranged at the base of the cells. At the mouth or duct of the gland the columnar cells give place to a squamous epithelium resembling that of the anterior urethra. The structure of the epithelium of these glands gives evidence of considerable functional activity, which function is the production of a rather viscid mucus. The purpose of this secretion is undoubtedly to lubricate and protect the anterior urethra. Because infections of these glands had been found only in adults between the ages of twenty and thirty-five years, it has been stated that they appear to reach their best development between these ages (1). In our clinic they have been found to occur in patients between the ages of twenty and forty-eight years inclusively. The fact that they do not apparently appear in ages under twenty remains to be explained. Possibly it may be due to a difference in the epithelial lining in very young patients. I have repeatedly examined the anterior urethras of young girls with chronic vaginitis, but have never succeeded in finding a suppurating focus in the urethra.

A great variety of organisms have been recovered from the human urethra (8): gonococcus, streptococcus, staphylococcus, diplococcus, colon bacillus, diphtheria group of bacilli, pneumococcus, smegma bacilli, typhoid and typhus, pseudogonococci (an organism morphologically like the gonococcus, but Gram-positive in reaction), and tuberculosis, and spirochaetae. The

Micrococcus catarrhalis (a Gram-negative diplococcus resembling the gonococcus) has also been found in the urethra (9); also in the vagina (10), from which locality it may easily gain entrance to the urethra. A Gram-negative diplococcus having the form of the gonococcus, but with variations in size, has been demonstrated in preparations from fresh cultures (11). In smears taken from apparently normal urethrae, near the external meatus, we have always found some bacteria. Typhoid, typhus, and tuberculosis gain entry by invasion from within during the general infection of these diseases. A primary tuberculosis urethritis has yet to be definitely demonstrated. The inclusion bodies of Lindner (8), believed by many authorities to be the bacterium causing trachoma, have been recovered from the female urethra, the newborn of which patient had a conjunctivitis at the time. This same organism can be isolated from the infant's conjunctiva if smears are taken during the early onset of the inflammation. Virus from the mother's urethra, or from the infant's conjunctiva, or from a case of true trachoma will, when applied to the eyes of a monkey or human adult, produce trachoma. It is only when the germ gains entrance to the eye of the adult that true trachoma arises. So often has this been noted and by so many different observers that trachoma has been considered a venereal disease.

Infections in the urethra may be pathogenic or non-pathogenic, and also pyogenic or non-pyogenic. Because of its great prevalence and the extent of its ravages, the gonococcus is the infection of greatest importance. From the suppurating discharge expressed from the urethra Gram-negative intracellular diplococci can occasionally be demonstrated. But there are a great number of cases in which a great number of bacteria can be recovered without showing any presence of this bacterium, at least in smears made directly from the discharge. Knowing that the character of the gonococcus changes more or less during long habitation in the tissues, and also that a great variety of flora follow in its wake, chronic suppurating cases may be considered probably of *Neisser* origin unless otherwise demonstrated. Such a type of case may be shown in the following laboratory reports taken from the record of the Stanford Women's Clinic:

No. 66510. July 22, 1918. Purulent-looking discharge expressed from urethral gland. Laboratory report of smear: Many pus cells and a few Gram-negative intracellular diplococci.

August 29, 1918. Purulent-looking discharge expressed from urethral gland. Laboratory report of smear: Many pus cells and epithelial cells and a few Gram-negative intracellular diplococci.

January 29, 1919. Purulent-looking discharge expressed from urethral gland. Laboratory report of smear: Many pus cells and Gram-negative and Gram-positive bacilli; no Gram-negative intracellular diplococci.

March 3, 1919. Purulent-looking discharge expressed from urethral gland. Laboratory report of smear: Many Gram-negative bacilli; no Gram-negative intracellular diplococci.

In subsequent smears no Gram-negative intracellular diplococci were found, although a purulent-looking discharge could be expressed every time the patient was examined. Such a case clearly indicates a great difficulty encountered in any bacteriological search for this bacterium.

In some cases Gram-negative, intracellular diplococci may be present in the urethra without giving rise to any urethral signs or symptoms, as illustrated in the following record:

No. 74194. Cervicitis. Thick, mucopurulent discharge from the cervix. No discharge from the urethra; anterior urethra clear. Laboratory report: Cervical smear: Many endothelial cells and a few Gram-positive bacilli. No Gram-negative intracellular diplococci seen. Smear from anterior urethra: Gram-negative intracellular diplococci. Many Gram-positive diplococci and bacilli.

What organism this is we cannot say at present. It appears that this bacterium is either in the incubation stage or else has remained here without producing any pathology.

B. coli infections producing suppurating para-urethral glands have been described by Fellner (12).

The changes in the urethra following pathogenic infections may be divided into two classes: those due to simple inflammation and those due to suppuration. In the former the lesion is like that of inflammation of a mucous membrane. These usually disappear in time or else yield to urinary antiseptics, except possibly some few chronic cases affecting the intermediate region. The squamous epithelium of the anterior urethra does not offer

proper soil for the invasion of bacteria. Luys (6) has described the changes due to gonorrhoeal infections. Tuberculosis (13) and syphilis (14, 15) have their specific lesions, which are described under these diseases.

The pyogenic infections are very important because of their frequency and chronicity. However, it should be noted that there can be expressed from some female urethras a material which is not one of suppuration. Sometimes a thick, creamy discharge, sometimes cheesy in consistency, can be expressed in which smegma bacilli have been found. At times one may be able to express a considerable amount of this material from the urethra. Occasionally a thin or thick white discharge is seen in the anterior urethra or can even be expressed directly from a gland, a material looking like that seen in the vagina at the time. This discharge has apparently passed from the latter place and lodged in the anterior urethra or gained access to a gland. The stained smears from each locality are alike. This condition is demonstrated in the following record:

No. 78639. Moderately thick whitish discharge in vagina. Moderately thick whitish discharge expressed from gland in floor of anterior urethra, right side. Smears made from each locality. Laboratory report: Urethral smear: Examination shows many epithelial cells which are squamous in type and have been denuded in masses. No pus cells nor lymphocytes seen. Many bacteria of various types, including large and small bacilli and small diplococci. Vaginal smear: Shows moderate number of squamous epithelial cells, a few lymphocytes, but no pus cells; numerous bacteria of various types, including large and small bacilli and small cocci occurring singly, in pairs, and in masses.

On two subsequent examinations at intervals of seven days, the same discharge was expressed from the same region of the urethra. Because the excretion resembles that seen in the vagina, macroscopically and microscopically, it is probable that the process was initiated by extension from the latter place or vice versa, and apparently does not extend beyond that of desquamation. In the chronic cases with suppurating infections we have a condition in which the original surface infection has disappeared; the bacteria have penetrated into the glands which offer the best soil for bacterial growth. In these minute

epithelial pockets chronic inflammation with suppuration may go on indefinitely and without a local symptom. At every examination of these patients one or more drops of pus can be expressed into the urethral meatus from these suppurating glands.

When the duct of a suppurating gland becomes occluded there will be formed a para-urethral abscess which may vary from the size of a marble to that of a large walnut. In the larger abscesses the mucous membrane and vascular capillaries have been destroyed in places and the process has extended into the adjacent tissue. In this way blood sometimes becomes mixed with the pus.

An important point which may be considered here is the way in which the discharge from the urethra may be carried up into the parturient tract of an obstetrical patient. In his description of the function of Skene's glands, namely, lubrication during coitus, Kelly has described a mechanism of displacement and eversion of the urethra which may be seen to take place when a finger is introduced into the vagina. At the first contact the labia urethrae are separated. This opens the urethral orifice. The tendency of the act of penetration is to displace the distal end of the urethra dorsally. As the vaginal wall becomes impinged upon, the displacement will become even more marked and the urethral orifice directed into the vagina. Thus during the examination of an obstetrical patient the urethral meatus tends to become directed toward the examining finger. Pressure on the anterior vaginal wall expresses any infected contents of the urethral glands, which are then carried up into the vagina by the penetrating finger. For this reason, unless one be exceedingly careful, a digital examination of an obstetrical patient had better be done per rectum. Also by this same mechanism infections may be carried to or into the urethral meatus. This is probably the way in which *B. coli* enter the urinary tract in the newly married, giving rise to what is called by Sippel (16) 'Kohabitation Cystitis und Pyelitis.' No matter how slight the abrasion of the urethral meatus which is directed into the vagina during the act of penetration, colon bacilli, which are

commonly present in this region, become rubbed in and thereby enter the urethral lymphatics and ascend. Some of these cases of *B. coli* infection disappear spontaneously; others lose their acute character, if they had any, and continue without symptoms, a chronic condition with acute exacerbations of pyelocystitis, commonly called by the laity 'bilious attacks.' This is also a probable way in which pyelitis of pregnancy develops.

Whenever a suppurating discharge can be expressed from the urethral glands I do not think it worth while to treat the infection with antiseptic instillation into the urethra or even directly into the ducts of the gland. Many of the cases so treated continue to discharge pus with little or no interruption. If the patient is kept under observation long enough, it will sometimes be found that what was thought to be cured was only pus-free for a time and has returned to its former suppurating condition.

Anterior urethral glands can easily be destroyed with the electric cauterizer and the suppurating condition terminated if the cauterization is sufficiently done. Whenever it is certain that the discharge expressed from the urethra is pus the anterior urethra should be cocaineized with a 10 per cent solution of cocaine and the skenoscope (17) introduced. Sometimes one may be able to see the entrance to the ducts. With a finger in the vagina and pressure applied along the urethra, pus may be seen to appear at one or two spots on the mucosa. In one instance, I saw as many as three drops appear at the same time in different places, and in a lateral position, not in the usual position on the floor of the urethra. At times one will be surprised to find that he is unable to express any discharge from a urethral gland, although some emerged from the meatus on examination before the instrument was introduced. This may be due to the fact that all of it had been expressed either from a gland or from the anterior urethra where it was lying. To tell whether or not it has come from a gland it is necessary to make the examination with the anterior urethra exposed so that the definite locality of any discharge may be seen. In case of failure to express any excretion for the reason just mentioned, the patient may be requested to return for another examination in a few days. The

instrument is then introduced for the preliminary search. The gland will be found to have filled again, especially so if pus had been found, and the discharge will be seen to emerge from its orifice on pressure. With a small wire a search is made in each drop of pus for the entrance to the duct into which the wire is passed as far as possible. This will act as a guide to direct the passage of a small wire electrocautery. Cauteize until as much tissue is destroyed as one thinks advisable. Failing to find the entrance to the duct one may cauterize the region with no guide other than the eye. If on a subsequent visit the treatment has been found to be unsuccessful or pus has appeared from another locality, the treatment should be repeated. I have never seen any ill effects follow this treatment and no recurrence after the second cauterization, which is usually more thoroughly done than the first one. Healing is very prompt. Also, I have never seen any suppurating gland further than a few millimeters from the meatus; never beyond easy reach of the cautery, and never more than one or two in number, on one occasion three. This fact, together with Keibel's and Mall's embryonic studies, leads me to believe that all of these cases are those of skenitis.

Para-urethral abscesses which cannot be emptied through the duct should be treated surgically by incision and drainage.

I wish to thank Dr. Frank Ellsworth Blaisdell for his help and assistance in examining the histological sections and for his contributions of microphotographs which accompany this paper. Thanks is also due others of my confreres whose interest has been an encouragement.

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PLATE 1

EXPLANATION OF FIGURES

1 Cross-section of a female urethra near meatus of a newborn baby. *G*, glands; *U*, urethral canal. Abundant squamous epithelium.

2 Higher magnification of a region on figure 1. All sections of this specimen show this type of epithelium.

3 Cross-section of the whole specimen of a female urethra near meatus of a nine months' baby. As disclosed in embryonic studies, the glandular budding is greater on the dorsal side.

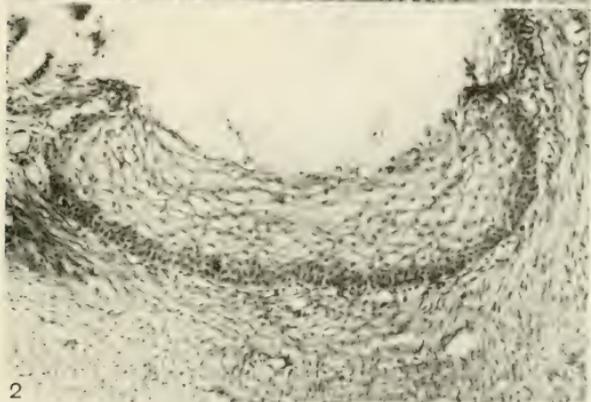


PLATE 2

EXPLANATION OF FIGURES

4 Cross-section of a female urethra near meatus of a nine months' baby; made from a region of figure 3. *G*, gland; *U*, urethral canal.

5 Cross-section of a female urethra near meatus of a twenty-three-year-old subject. Epithelium is squamous. This is undoubtedly a Skene's gland. *U*, urethral canal; *D*, duct; *G*, gland.

6 Higher magnification of a region on figure 5, but made $\frac{5}{100}$ mm. posterior to the section made of the latter. This section shows columnar epithelium of the urethral canal, *U*, and the gland, *G*.

7 Cross-section of a female urethra near meatus of a forty-three-year-old subject. *G*, gland; *E*, epithelium; *U*, urethral canal.

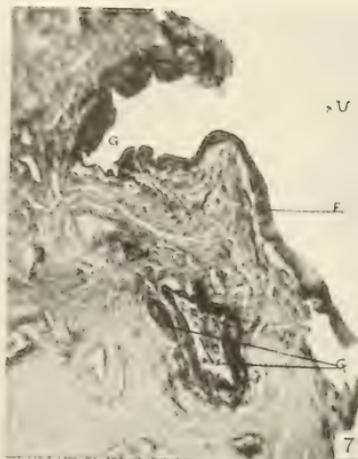
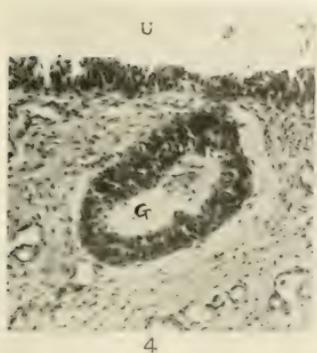


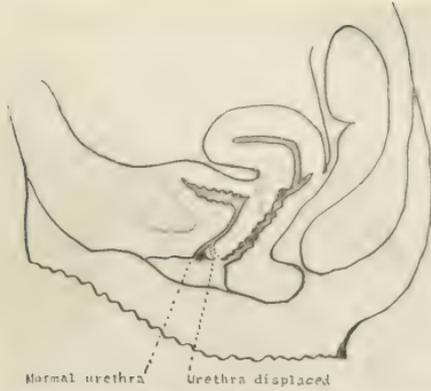
PLATE 3

EXPLANATION OF FIGURES

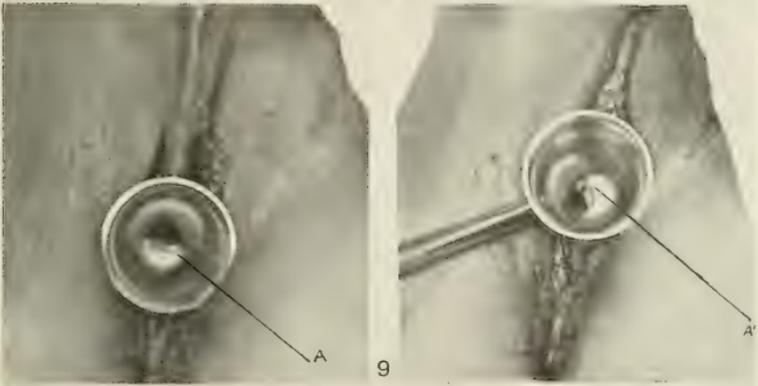
8 Drawing of a sagittal section through the center of a female pelvis representing the urethral displacement on vaginal penetration.

9 Instrument in position for examination and treatment. Exposure of drops of pus (*A*) in different localities of the urethra in different subjects.

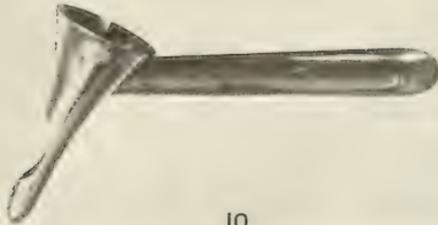
10 Skenoscope.



Normal urethra Urethra displaced
8



9



10

Resumen por el autor, Richard E. Scammon,
Universidad de Minnesota.

Un sencillo aparato de calcar para hacer reconstrucciones
topográficas.

Este aparato ha sido ideado para hacer reconstrucciones gráficas de fetos y otros objetos pequeños. Consiste esencialmente de dos partes: Una placa de vidrio cuadriculada, cuyas líneas distan entre sí un centímetro, colocada sobre un tablero, y un ocular con un eje óptico establecido mediante un pequeño orificio superior y una cruz formada por dos cerdas cruzadas, situada inferiormente.

En la base del ocular se ha cortado un cuadrante para permitir la orientación de aquel con referencia a las líneas de la cuadrícula. Los bordes del cuadrante son biselados y divididos en una escala milimétrica para medir pequeñas distancias. Los ejemplares que se desea reconstruir se colocan sobre el tablero de la base, y después de orientarlos con referencia a la línea basal de la cuadrícula dividida en centímetros, se dibujan en papel cuadriculado por medio de una serie de lecturas sucesivas tomadas con el ocular.

Translation by José F. Nonidez
Cornell Medical College, New York

A SIMPLE TRACING APPARATUS FOR MAKING TOPOGRAPHIC RECONSTRUCTIONS

RICHARD E. SCAMMON

Institute of Anatomy, University of Minnesota

THREE FIGURES

The study of certain phases of the anatomy of the fetus and infant is hindered somewhat by the lack of any simple method of making topographic reconstructions of the various organs and regions. Graphic reconstruction from microscopic sections, which is so successful in embryologic work, is generally impracticable here, for the preparation of even a few sets of serial sections of this material requires an almost prohibitive amount of time and labor, and the thorough decalcification necessary for the larger specimens usually causes serious shrinkage and distortion in the process of embedding. The usual methods employed in the study of adult topography are also inadequate for this work. Topographic reconstructions of the adult are generally made either by graphic reconstruction from free-hand transverse sections after the method first suggested by Henke or by plotting from fixed points which are established by setting long pins or skewers in the body in certain definite positions before dissection is begun. But to make accurate reconstructions of the smaller structures of the fetus and infant the sections must be cut so thin that they are extremely fragile and subject to distortion. And the fixed point method is very inconvenient both because of the difficulty in placing the pins firmly in position in the delicate tissues and because these pins make the subsequent dissection of the smaller regions almost impossible.

The apparatus which is described here was devised to overcome some of these difficulties, and after a considerable trial has been found sufficiently useful to warrant the publication of

an account of it. It consists essentially of a tracing stand, covered by a glass grating, and an eyepiece.

The stand is shown in figure 1. Its base is a slab of hardwood, 25 inches long, 17 inches wide, and 1.5 inches thick. Seven inches above this base is a sheet of heavy plate glass inclosed in a strong hardwood frame, which is supported at its corners by four brass rods. At one end these rods or legs are connected with the frame by hinges and firmly attached to the base-board

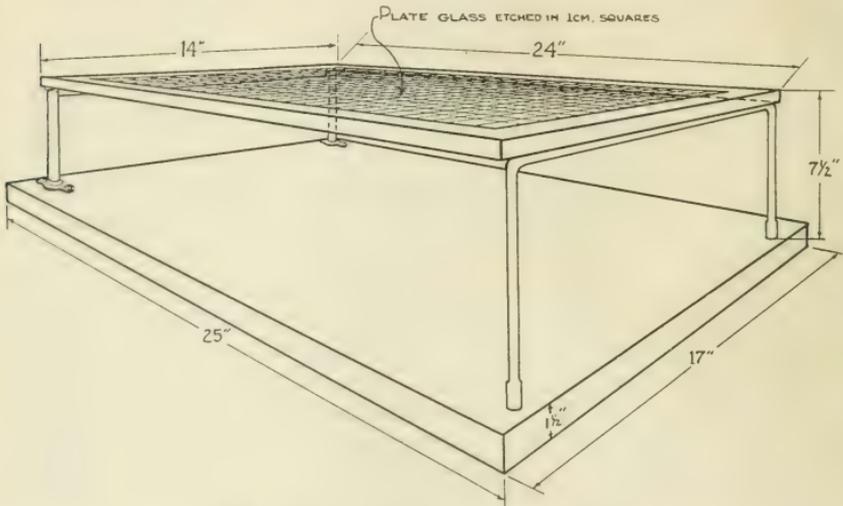


Fig. 1 Stand of reconstruction apparatus.

with screws. At the other their upper ends are screwed to the frame of the plate, but their lower ends are covered by rubber caps which rest freely on the base-board. This permits the frame to be raised so that large objects may be easily placed on the base-board below.

The glass plate is ruled with a centimeter grating and the lines of this grating are numbered or lettered consecutively at its margin. The middle longitudinal and the middle cross line of the grating are ruled a little heavier than the others and are filled with pigment to distinguish them as base lines (fig. 3).

The eyepiece is a brass tube 4 inches long and 0.8 inch in diameter (fig. 2, *A*). Its upper end is closed by a screw cap which contains a central pinhole opening (fig. 2, *B*). At the bottom of the tube are cross-hairs of spun glass or very fine wire which are set a little above its lower opening and cross in the optical

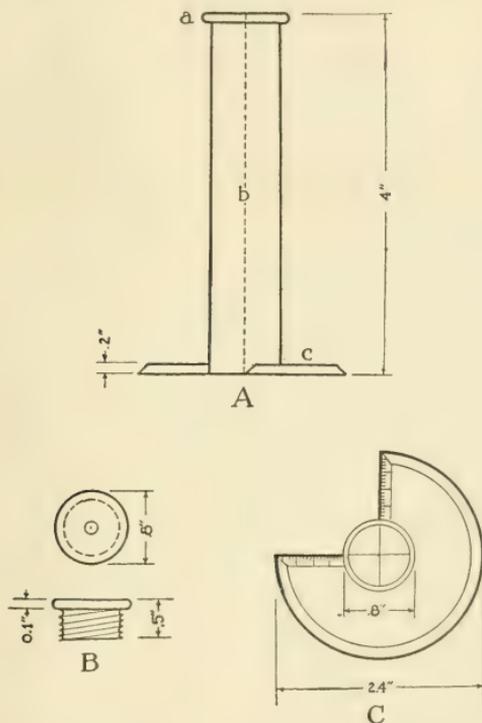


Fig. 2 Eyepiece of reconstruction apparatus. *A*, entire eyepiece; *B*, detail of screw cap with pinhole opening; *C*, detail of quadrant base.

axis of the tube directly in line with the pinhole opening in the cap. The lower end of the tube is set in the center of a circular plate of brass 2.4 inches in diameter and 0.2 inch thick. One quadrant of this base is cut away, its margins being so adjusted that they fall directly in line with the cross-hairs of the eyepiece.

The edges of the quadrant are beveled and are graduated in millimeters, the zero points of the scales lying exactly 1 cm. from the optical center of the eyepiece (fig. 2, *C*).

The method of using the apparatus is simple. The specimen to be reconstructed is fixed firmly in a tray or better set in a base of plaster of Paris or hard wax. It is then placed on the base-board and adjusted so that its midline corresponds approximately with the midline of the grating on the glass plate above it. Orientation points are then established by marking the specimen with dots of indelible ink or by setting small pins in it. At least three such points should be established as far apart as possible and in regions which will not be disturbed in the course of the subsequent dissection. A large sheet of coördinate paper is now numbered to correspond with the numbering of the grating, and base lines corresponding to those of the grating are drawn upon it. The exact position of the orientation points and the outlines and superficial landmarks of the specimen are now determined by successive readings with the eyepiece which is passed over the grating. As these determinations are made they are recorded in their proper places on the coördinate paper, and the first plot giving the outlines of the specimen is completed by connecting these points. After the outline is made the specimen may be dissected layer by layer and as the different structures are exposed they may be outlined in their proper positions on the plot by replacing the specimen under the grating, adjusting the orientation points to their recorded positions, and taking the necessary readings with the eyepiece. With a little practice this process can be carried out quite rapidly. Readings with the eyepiece to half-centimeters can be made directly from the lines of the grating and readings to half-millimeters by using the scales on the margins of the quadrant. The specimen should be strongly illuminated when the readings are made. Orthographic projection is assured by the use of the eyepiece with a vertical optical axis established by the pinhole opening and cross-hairs. It is possible to make the reconstruction at any magnification desired by modifying the scale of the coördinate paper.

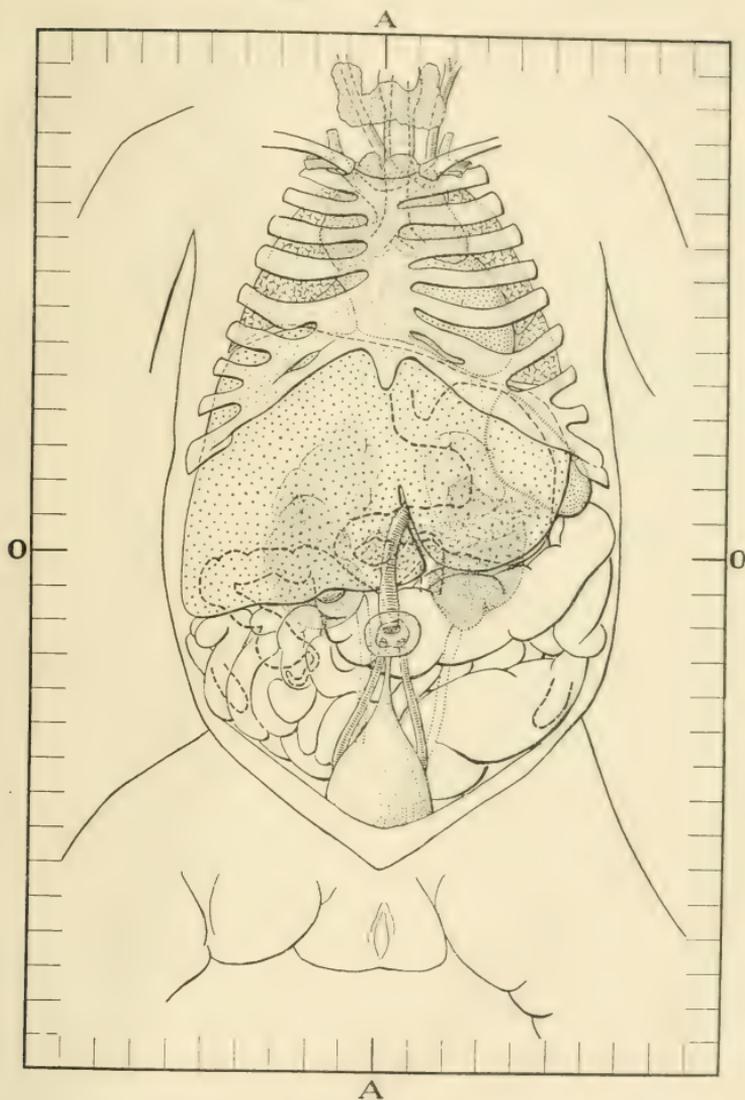
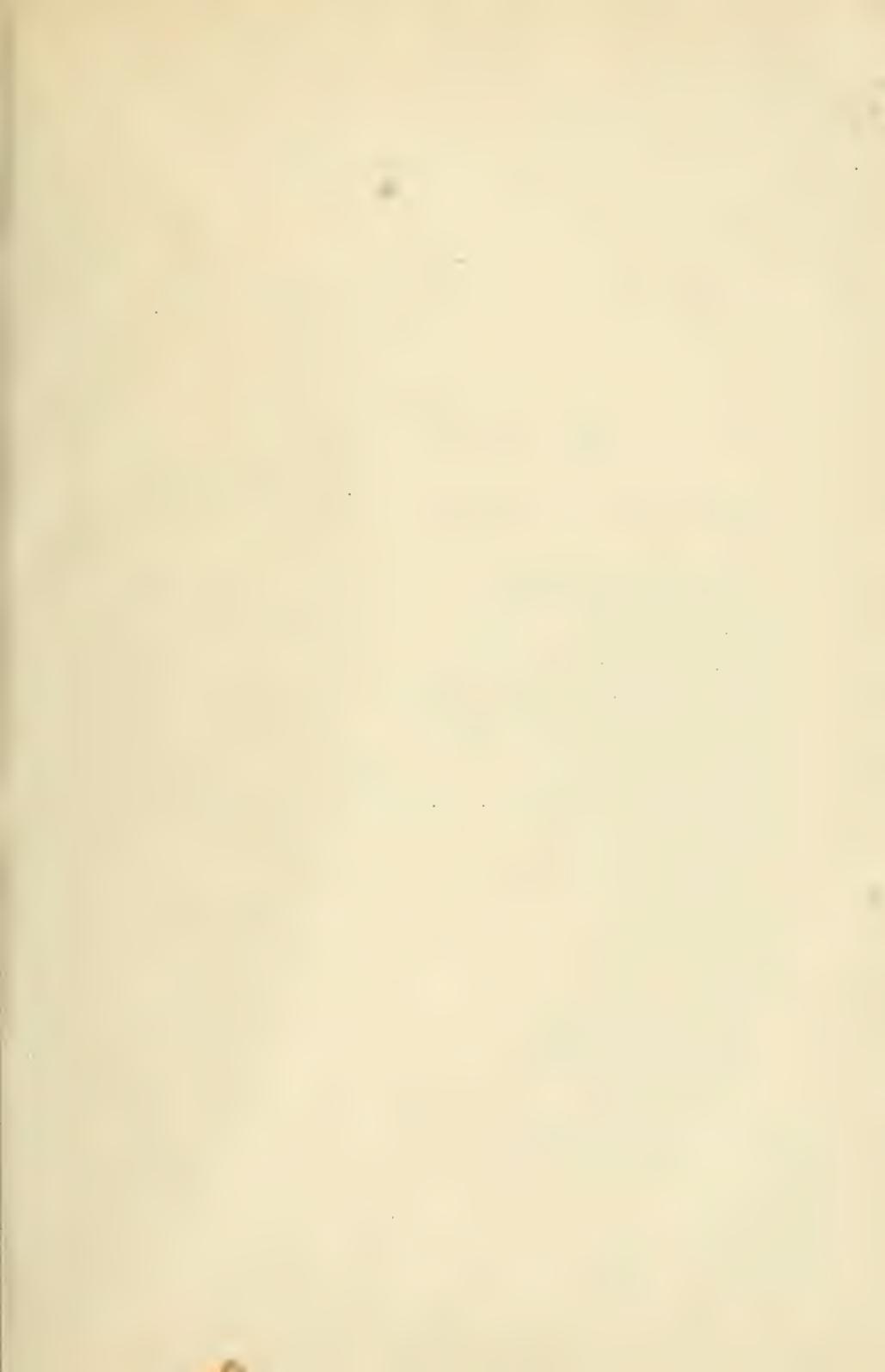


Fig. 3 Reconstruction of the abdominal and thoracic viscera of a full-term newborn infant. Made by H. J. Bower and W. C. Stillwell with the apparatus herein described. The reconstruction has been retraced and reduced to one-half the original (natural) size. The centimeter scale of the grating is shown at the margins of the drawing. *A-A and O-O are the longitudinal and cross base-lines.

The chief sources of error in making reconstructions of this kind are due, first, to changes in the form of the specimen which may occur in the course of dissection and, second, to variations caused by the improper adjustment of the eyepiece. The first may be avoided, in a great measure, by partially embedding the specimen in a firm base of plaster or wax as mentioned above and by care in dissection. The second can be entirely eliminated if care is taken to see that the margins of the quadrant are either parallel or at right angles to the lines of the grating before each reading is made.

An example of a reconstruction by this method of the thoracic and abdominal viscera of a full-term stillborn infant is shown in figure 3. The original plotting has been retraced and inked, the published figure being one-half the size of the original.



Resumen por el autor, Richard E. Scammon,
Universidad de Minnesota.

Nota sobre la relación entre el peso de la tiroides y el del timo
en el hombre.

La variación de peso del timo y la tiroides en adultos jóvenes, en apariencia normales (determinados por los datos de Dustin y Zunz) es muy grande. Los pesos de estos órganos demuestran la existencia de una correlación ligeramente negativa en la madurez temprana. Los pesos del timo y la tiroides del recién nacido varían también considerablemente, pero presentan una ligera correlación positiva. Las conclusiones de Dustin y Zunz sobre el valor de estos datos como prueba de una correlación funcional entre el timo y la tiroides no reciben confirmación en los estudios del autor.

Translation by José F. Nonidez
Cornell Medical College, New York

A NOTE ON THE RELATION BETWEEN THE WEIGHT OF THE THYROID AND THE WEIGHT OF THE THYMUS IN MAN

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In a recent publication Dustin and Zunz (1) have recorded some interesting observations on the weight of the thyroid and the thymus in early maturity. Their data are quite unique, consisting of weighings of the thyroids and thymi of thirty-eight individuals who, with one exception, died within forty-eight hours after receiving wounds in battle, and who came to autopsy within twelve hours or less after death. We are thus furnished with a series of records of the weight of the thymus in presumably normal young adults which greatly improves our knowledge of the later ponderal changes of this organ.

Dustin and Zunz have analyzed this material by means of tabulations and a graph in which the thymus weight is plotted against the thyroid weight. They found a negative correlation between thymus weight and thyroid weight in man, and they conclude that their results support the experimental findings of Gley (2) and others who have observed an increase in the thymus following thyroidectomy in amphibia.

Although this series of cases is very small for any statistical study, its unique character and the importance of the conclusions which have been drawn from its examination seem to warrant its further analysis by some of the simpler biometric methods. Accordingly, the standard deviation and the coefficient of variation have been determined for the thymus and thyroid in the series and also the coefficient of correlation between the two organs. These determinations were first made for the entire series of cases as given in the original article, and second for the same series with the omission of four cases which seem to be of doubtful value.

In the complete series the average weight of the thyroid is 32.0 ± 2.4 grams, the standard deviation 21.8 grams, and the coefficient of variation 0.68. The average thymus weight is 15.6 ± 0.7 grams, the standard deviation 7.07 grams and the coefficient of variation 0.45. The coefficient of correlation between the thyroid and the thymus is -0.265 with a probable error of ± 0.102 .

The second calculation was made from the series after the omission of the following cases.

Case 1, in which the weight of the thyroid was 134.54 grams, nearly four times the average weight of the group and over two times the weight of the next member of the series. It can scarcely be doubted that this great enlargement was associated with thyroid disease.

Cases 2 and 11, in which there was a complete involution of the thymus. As these cases were individuals aged twenty-five and twenty-eight years, respectively, it is most probable that the thymi had undergone accidental involution, presumably in some previous illness.

Case 38 was a youth but fourteen years old and cannot be properly included with a series of young adults, since the thymus undergoes profound weight changes in adolescence.

In the selected series, with these four cases omitted, the average thyroid weight was 26.6 ± 1.41 grams, the standard deviation 12.26 grams, and the coefficient of variation 0.45. The average weight of the thymus was 16.2 ± 0.64 grams, the standard deviation 5.49 grams, and the coefficient of variation 0.33. The coefficient of correlation of the thyroid and thymus was -0.156 with a probable error of ± 0.107 .

These calculations show a negative correlation between the weight of the thyroid and the weight of the thymus in the complete series, as Dustin and Zunz suspected. But this correlation is so low and the probable error is so large that we are hardly justified in attaching any particular significance to it. This seems the more probable since when the four cases which are of very doubtful value are omitted the correlation drops to from -0.265 to -0.156 and the probable error remains almost un-

changed. A slight negative correlation might well exist between the two organs, since the thyroid follows the scheme of general body growth and increases a little in weight during the third decade, while the thymus decreases in absolute as well as relative weight after early adolescence. But this negative correlation does not warrant the assumption of a functional relation between the two organs; a similar correlation might be expected between the thymus and any of the viscera which follow the general scheme of the growth in mass of the body as a whole.

In order to test this relation in another way I have calculated the coefficient of correlation of the thyroid and thymus in a series of twenty-five full-term newborn children. The data for this series were taken in part from the lists of cases reported by Valtorta (3) and Lomer (4) and in part from my own records. The average weight of the thyroid in this series was 3.4 ± 0.24 grams, the standard deviation 1.8 grams, and the coefficient of variation 0.53. The average thymus weight was 14.2 ± 0.90 grams, the standard deviation 6.7 grams, and the coefficient of variation 0.47. The coefficient of correlation of the thymus and thyroid was +0.19 with a probable error of ± 0.08 . Thus in the newborn, as in the adult, the variability of the thymus and the thyroid is very great and the correlation between the two organs is quite small. But, in contrast to the adult, the slight correlation which does exist in the newborn is a positive one.

These figures indicate that any correlation which may exist between the weights of the thyroid and the thymus is inconstant in postnatal life, and they offer little if any support to the concept of a direct functional relation between the two organs.

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- 4 LOMER 1889 Ueber Gewichtsbestimmung der einzelnen Organe Neugeborener. *Zeitschr. f. Geburtsh. u. Gynäkol.*, Bd. 16, S. 106-130.

Resumen por el autor, George B. Wislocki,
Johns Hopkins Medical School.

Observaciones sobre el comportamiento de la tinta china inyectada en animales durante la preñez.

Experimentos llevados a cabo durante muchos años han venido a demostrar que las partículas inertes que flotan en la circulación materna no pueden atravesar la placenta y penetrar en el feto. La explicación de este fenómeno, mediante el cual el material en suspensión no atraviesa la placenta o las membranas fetales, no ha sido hallada más que para un grupo de substancias, esto es, los colorantes vitales. Estos colorantes, conforme Goldmann ha demostrado en el caso de la rata y ratón, son absorbidos y acumulados en el epitelio coriónico y las células de la membrana vitelina, y de este modo se previene su penetración en el feto. El autor ha inyectado gránulos de carbón suspendidos en acacia (tinta china) en una serie de conejillos de indias, conejos, gatos y perros preñados. Los animales fueron sacrificados después de unos cuantos días y los tejidos examinados. El autor ha podido observar que los gránulos de carbón se depositan en el hígado, bazo, pulmones y médula ósea de la madre. En la placenta, membranas fetales y órganos de los fetos no pudo hallar carbón, ni aún estudiándolos bajo el microscopio. La conclusión que se deriva de la repulsión de las partículas de tinta por las células de la placenta y membranas fetales es que dichas células son incapaces de absorber o fagocitar material extraño de tamaño grosero, flotante en la sangre. El límite del tamaño de las partículas que pueden absorber debe estar localizado entre el de una suspensión grosera, tal como la tinta china, y una dispersión ultramicroscópica, como el azul trypan.

Translation by José F. Nonidez
Cornell Medical College, New York

OBSERVATIONS UPON THE BEHAVIOR OF CARBON GRANULES INJECTED INTO PREGNANT ANIMALS

GEORGE B. WISLOCKI

Department of Anatomy, Johns Hopkins Medical School

It has long been known that certain cells of the body possess the power of removing foreign particulate matter from the blood-stream. Thus, when foreign particles, such as the carbon granules of india ink, are injected into the circulation of an animal, they are completely removed from the blood-stream in a remarkably short period of time. Gross and microscopic examination of the tissues of the animal reveals that the endothelial cells in certain organs have removed the ink from the circulation. The endothelial cells lining the sinusoidal channels of the liver and spleen are found heavily laden with the foreign particles. In addition to the granules of carbon which have been actually phagocytized, aggregations of the particulate matter into tiny clumps are found within the lumen of the blood sinuses. The bone-marrow in many animals is the site of a similar but subordinate process.

The question of the behavior of the cells of the placenta toward particulate matter circulating in the blood-stream has been only incompletely investigated. Recent writers on the problem of placental transmission, namely, Zuntz ('04), Kehrer ('08), and Hofbauer ('10), concur in the statement that particulate matter does not pass from the maternal into the fetal blood-stream. The explanation of the failure of suspended material to pass through the placenta or fetal membranes has not been given except for one group of substances, namely, the vital dyes. The vital dyes, which are ultramicroscopic dispersions of certain of the acid azo dyes have been rather fully investigated by Goldmann ('09). He showed that when these dyes are introduced into the blood-stream of a pregnant mouse or rat,

they stain the maternal tissues, the placenta, and the outermost fetal membrane, but fail to stain the fetus. He discovered that the reason for this apparently was that the dye-stuffs were absorbed and stored by the cells of the chorion and vitelline membrane and thereby prevented from entering the fetus.

It has, however, not been determined whether more coarsely dispersed substances than the 'vital dyes,' such as the carbon granules of india ink, are similarly phagocytized and stored by the cells of the placenta and fetal membranes or whether they are completely rejected by these cells. In the literature one finds numerous brief statements regarding the fate of coarse particulate substances injected into the blood-stream of pregnant animals. The earlier experimental work is surprisingly contradictory and but little importance can be assigned to most of it, as the observations were made on an insufficient number of animals and the methods employed were often open to criticism. Thus, Reitz ('68) described cinnabar (red sulphide of mercury) in the tissues of a rabbit fetus after injecting the mother; Caspary ('77) reported a similar result with cinnabar in a rabbit; Perls ('77) recorded the passage of cinnabar and ultramarine into the fetuses in several rabbits and dogs; Mars ('80) observed the passage of a number of emulsified substances into rabbit fetuses, and, finally, Pyle ('84) stated that he observed the passage of ultramarine into a series of rabbit fetuses.

On the other hand, Hoffmann and Langan ('69) failed to find cinnabar in the fetuses of a rabbit which they injected; Fehling ('77) and Ahlfeld ('77) reported the failure to find india ink in the liver, kidneys, or blood of the fetuses of several rabbits, and Miropolsky ('85) obtained similar negative results with cinnabar.

Krukenberg ('88), who can be said to have undertaken the first thorough investigation of this kind, injected a suspension of barium sulphate into one series of pregnant rabbits and a non-pathogenic organism, *B. prodigiosus*, into another series. Neither particles of barium sulphate nor organisms were recoverable from the tissues of the fetuses. His experiments left little doubt that particulate materials, such as he employed, are not transmitted from mother to fetus.

Hofbauer ('10) obtained similar results after injecting colloidal silver and silicium into several pregnant animals. Neither of the substances could be found in the fetal organs.

Finally, Goldmann ('09) undertook his experiments with acid azo dyes, and showed that they, too, were not transmitted to the fetus. Goldmann's report contains the only description of the microscopic examination of the placenta and fetal membranes. In the mouse and rat the giant-cells, the chorionic ectoderm of the labyrinth, and the epithelium of the vitelline membrane were found heavily laden with granules of dye; these cellular accumulations of the dye-stuff were looked upon as evidencing the protective mechanisms of the fetus.

In the present experiments a filtered solution of india ink was used. The ink was administered intravenously to a series of pregnant animals, consisting of one dog, three cats, three rabbits, and three guinea-pigs. The amount of ink injected was regulated according to the size of the animals, the guinea-pigs receiving 1 cc., the rabbits and cats 5 cc., the dog 15 cc., on two successive days. The animals were sacrificed one or two days after the last injection.

The gross distribution of the india ink was essentially the same in all the species of animals examined. At autopsy the liver and spleen were found to be deep black and the lungs presented a grayish appearance. The bone-marrow in the rabbit was of the same color as the liver and spleen; in the guinea-pig it was not so black, while in the cat and dog its color appeared to be normal. The uterus appeared unstained. The placentae, fetal membranes, and the fetuses showed nothing to the naked eye suggesting the presence of ink particles in these tissues. The remaining organs and tissues of the animals appeared normal. The findings in gross, therefore, indicated that the injected ink granules had in whole or greater part been segregated in the liver, spleen, bone-marrow, and lungs, and that none had been deposited in the placentae, fetal membranes, or fetuses.

Microscopic examination revealed the characteristic deposition of the carbon particles in the endothelial phagocytes of the liver, spleen, and bone-marrow. Particles, in part free and in part

phagocytized, were also found, to a slight extent, in the inter-alveolar septa of the lungs. In the remaining organs and tissues of the body, with the exception of a few particles occasionally caught in a blood vessel or phagocytized within an endothelial cell, the carbon granules of the india ink were conspicuously absent.

No carbon particles could be found on examining the placentae. The chorionic epithelium, which in varying patterns is the predominating tissue in them all, showed no evidence of having absorbed particles of ink. The endothelial cells, which in the cat's and dog's placentae completely line the maternal vessels, had not the power of phagocytizing ink particles as had the endothelium of the liver, spleen, and bone-marrow. None of the ink-particles had agglutinated in either the maternal vessels of the dog's and cat's placentae, or in the corresponding sinuses of the rabbit's and guinea-pig's placentae, as they do in the sinuses of the liver and spleen. In the columnar cells of the chorion which flank the placentae of the dog and cat, known respectively as the 'green' and 'brown borders,' no carbon was visible. Nor were particles of ink discovered in the cells covering the vitelline membrane which in the rabbit and guinea-pig forms the outermost fetal membrane and faces the uterine mucosa.

The absence of particles of india ink in all these localities is surprising, since in vitally stained animals these same cells, namely, the chorionic epithelium and the epithelium of the vitelline membrane, are heavily laden with minute granules of trypan blue.

The conclusion to be drawn from the rejection of the ink particles by the cells of the placentae and fetal membranes is that they are incapable of absorbing or phagocytizing coarse, foreign particulate matter afloat in the blood-stream. The limit of the size of particles which they are capable of accepting must lie somewhere between that of a coarse suspension, such as india ink, and an ultramicroscopic dispersion, such as trypan blue. Trypan blue in turn, although absorbed by the chorionic epithelium and fetal membranes, is incapable of entering the

fetal circulation as true solutions have been shown readily to do. Trypan blue, however, may be on the border line of transmissibility, since traces of it actually enter the fetal circulation in the rabbit and guinea-pig.

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THIRTY-SEVENTH SESSION

Wistar Institute of Anatomy and Biology, Philadelphia
March 24, 25 and 26, 1921

THURSDAY, MARCH 24, 9.30 A.M.

The Thirty-seventh Session of the American Association of Anatomists was called to order by President Charles F. W. McClure, who appointed the following committees:

Committee on Nominations for 1921: Professor Ross G. Harrison, Chairman, and Professors Henry H. Donaldson and G. Carl Huber.

Auditing Committee: Professor F. T. Lewis, chairman, and Professor Stacy R. Guild.

The remaining morning session was devoted to the presentation of scientific papers.

FRIDAY, MARCH 25, 11.30 A.M. ASSOCIATION BUSINESS MEETING, President CHARLES F. W. McCLURE, presiding.

The Secretary reported that the minutes of the Thirty-sixth Session were printed in full in *The Anatomical Record*, volume 18, number 3, pages 211 to 218. On motion, seconded and carried, the minutes of the Thirty-sixth Session were approved by the Association as printed in *The Anatomical Record*.

Professor F. T. Lewis reported for the Auditing Committee as follows: The undersigned Auditing Committee has examined the accounts of Doctor Charles R. Stockard, Secretary-Treasurer of the Association of Anatomists, and finds the same to be correct with proper vouchers for expenditures and bank balance on December 29, 1920, of \$164.40.

[Signed] F. T. LEWIS,
STACY R. GUILD

The Treasurer made the following report for the year 1920:

Balance on hand January 20, 1920, when accounts were last audited	\$173.12
Receipts from dues 1920	2,521.43
	<hr/>
Total deposits	\$2,694.55
Expenditures for 1920:	
Expenses Secretary-Treasurer, Washington Meeting	\$36.08
Postage and Telegrams	44.90
Printing and Stationery	160.75
Collection and exchange on drafts	3.67
Stenography, typewriting	48.75
Wistar Institute, subscriptions to Journal of Anatomy, Anatomical Record, etc	2,236.00
	<hr/>
Total expenditures	\$2,530.15
	<hr/>
Balance on hand	\$164.40
Balance on hand deposited in the name of the American Association of Anatomists in the Corn Exchange Bank, New York City.	

On motion the report of the Auditing Committee and the Treasurer were accepted and adopted.

The Committee on Nominations, through its Chairman, Professor H. H. Donaldson, placed before the Association the following names for members of the Executive Committee, term expiring 1924, Professors S. W. Ranson and R. J. Terry.

On motion the Secretary was instructed to cast a ballot for the election of the above named.

The Secretary presented the following names recommended by the Executive Committee for election to membership in the American Association of Anatomists:

- ABBOTT, MAUDE E., A.B., C.M., M.D., Curator of the *Medical Museum, McGill University, Montreal, Canada.*
- ALLEN, EDGAR, Ph.B., A.M., Instructor in Anatomy, Washington University School of Medicine, *4555 McKinley Avenue, St. Louis, Mo.*
- ALFORD, LELAND BARTON, A.B., M.D., Associate in Clinical Neurology, Washington University School of Medicine, *Humboldt Building, St. Louis, Mo.*
- BLAIR, VILRAY PAPIN, A.M., M.D., Associate in Clinical Surgery, Washington University School of Medicine, *Metropolitan Building, St. Louis, Mo.*
- BROOKS, BARNEY, B.S., M.D., Associate in Clinical Surgery, Washington University School of Medicine, *4918 Forest Park Boulevard, St. Louis, Mo.*

- DART, RAYMOND A., M.B., Ch.M., M.Sc., Demonstrator in Anatomy, University College, Gower St., London, W. C. 1, England. *Temporary Address: Johns Hopkins Medical School, Baltimore, Md.*
- DAVIS, WARREN B., M.D., Instructor in Anatomy, Jefferson Medical College, 135 S. 18th Street, Philadelphia, Pa.
- DE CARLO, JOHN, M.D., Instructor in Topographic and Applied Anatomy, Jefferson Medical College, 1124 Ellsworth St., Philadelphia, Pa.
- DENDY, ARTHUR, D.Sc., F.R.S., Professor of Zoology, University of London, King's College, Strand W. C., London, England.
- GARCIA, ARTURO, A.B., M.D., Professor of Anatomy, College of Medicine and Surgery, Manila, Philippine Islands.
- GRAVES, WILLIAM W., M.D., Professor of Nervous and Mental Diseases, St. Louis University School of Medicine, Metropolitan Building, St. Louis, Mo.
- GREGORY, WILLIAM KING, A.M., Ph.D., Curator of Comparative Anatomy, American Museum of Natural History, 77th Street and Central Park West, New York City.
- GEORGE, WESLEY CRITZ, A.M., Ph.D., Associate Professor of Histology and Embryology, University of North Carolina Medical School, Chapel Hill, North Carolina.
- HARTMAN, CARL G., Ph.D., Associate Professor of Zoology, University of Texas, Austin, Texas.
- HAUSMAN, LOUIS, A.B., M.D., Instructor in Psychiatry, Johns Hopkins Hospital, Baltimore, Md.
- HILL, EBEN CLAYTON, A.B., M.D., Instructor in Anatomy, Johns Hopkins Medical School, Baltimore, Md.
- HUGHSON, WALTER, S.B., M.D., Assistant in Anatomy, Johns Hopkins Medical School, Baltimore, Md.
- INOUE, MICHIO, M.D., Professor of Anatomy, Tokyo Imperial University, Tokyo, Japan.
- LEVI, GIUSEPPE, M.D., Professor of Anatomy, University of Torino, Torino, Italy.
- MEAKER, SAMUEL R., A.B., M.D., Teaching Fellow, Department of Anatomy, Harvard Medical School, Boston, Mass.
- NANAGAS, JUAN CANCIA, M.D., Assistant Professor of Anatomy, College of Medicine and Surgery, Manila, Philippine Islands. (*Temporary address—Dept. of Anatomy, Johns Hopkins Medical School, Baltimore.*)
- NICHOLAS, JOHN SPANGLER, B.S., M.S., University Fellow in Zoology, Osborn Zoological Laboratory, Yale University, New Haven, Conn.
- NONIDIZ, JOSÉ F., Sc.M., Sc.D., Instructor in Anatomy, Cornell University Medical College, 1st Avenue and 28th Street, New York City.
- PATTEN, BRADLEY MERRILL, A.M., Ph.D., Assistant Professor of Histology and Embryology, School of Medicine, Western Reserve University, 1353 East 9th Street, Cleveland, Ohio.
- PERKINS, ORMAN C., A.M., Assistant Professor of Anatomy, Long Island College Hospital, 335 Henry St., Brooklyn, New York.
- SACHS, ERNEST, A.B., M.D., Professor of Clinical and Neurological Surgery, Washington University School of Medicine, 97 Arundel Place, St. Louis, Mo.
- SHELLSHEAR, JOSEPH LEXDEN, M.B., Ch.M., Demonstrator of Anatomy, University College, Gower St., London, W. C. 1, England. (*Present address—Dept. of Anatomy, Johns Hopkins Medical School, Baltimore.*)

- SMITH, DAVID T., A.B., Medical Student, *Johns Hopkins Medical School, Baltimore, Md.*
- STONE, LEON STANSFIELD, Ph.B., Assistant in Anatomy, *Medical College, Yale University, New Haven, Conn.*
- STONE, ROBERT S., B.A., Assistant in Anatomy, *Peking Union Medical College, Peking, China.*
- STOPFORD, JOHN SEBASTIAN B., M.D., Professor of Anatomy, *University of Manchester, Manchester, England.*
- SWINGLE, W. W., Ph.D., Instructor in Zoology, *Yale University, New Haven, Conn.*
- VAN DER HORST, C. J., Ph.D., *Zoologisch Laboratorium, Pl. Muidergracht 34, Amsterdam, Holland.*
- WALMSLEY, THOMAS, M.D., Professor of Anatomy, *Queens University of Belfast, Belfast, Ireland.*
- WOOLLARD, HERBERT T., M.D., Demonstrator of Anatomy, *University College, Gower St., London, W. C. 1, England.*

On motion, the Secretary was instructed to cast a ballot for all the candidates proposed by the Executive Committee. Carried.

The Secretary then announced the following names as dropped from the list of members on account of non-payment of dues for the past two years:

- DR. A. E. AMSBAUGH, *Letterman Hospital, San Francisco.*
DR. ROBERT S. GUTSELL, *University of Minnesota.*
DR. JOHN A. KITTLESON, *University of Nebraska, Omaha.*
DR. WILLIAM E. McCORMACK, *University of Louisville.*
DR. GEORGE WALKER, *Johns Hopkins Medical School.*

It was announced that the Executive Committee had voted to hold the next annual meeting at Yale University, New Haven, Conn., during the last week of December, 1921. The Federation of Biological Societies holds its meeting in New Haven at the same time.

A Committee on Editorship of Journals was elected by the Executive Committee following the last meeting: C. R. Stockard, Chairman; C. M. Jackson, G. L. Streeter, R. J. Terry and C. R. Bardeen.

The Committee on Editorship of Journals reported as follows:

PROPOSED ORGANIZATION OF A JOURNAL COMMITTEE
OF THE AMERICAN ASSOCIATION OF ANATOMISTS
AND ITS DUTIES.

1. There shall be organized a Journal Committee composed of five members elected by the Association.

2. The Committee shall be established in 1921, as follows: Ten members of the Association shall be nominated by the Executive Committee of the Association. Additional nominations may be made from the floor. Members of the Advisory Board of The Wistar Institute shall not be eligible for nomination in 1921. Election shall be by ballot. The five receiving the largest number of ballots shall constitute the Committee. In case of a tie, the choice of those thus tying shall be by lot. Of the five thus chosen, the one receiving the greatest number of votes shall serve for five years, the next for four years, the next for three years, the next for two years and the next for one year.

3. At the annual meeting in 1922, and subsequent years, one member shall be elected to serve for five years. Members are eligible for reëlection. At least two nominations shall be made by the Executive Committee of the Association and other nominations may be made from the floor. The election shall be by ballot.

4. In case of resignation of a member of the committee, the place of the member thus resigning may be filled temporarily by appointment by the Committee itself until the next annual meeting of the society. At this meeting the place vacated shall be filled by nomination and ballot as outlined in Section 3, except that the election shall be for the balance of the unfulfilled term.

5. The duties of the Committee shall be:

(a) The selection of a responsible Editor for The American Journal of Anatomy and of a responsible Editor for The Anatomical Record.

(b) The appointment of Associate Editors, if such are desirable, shall be made by the committee in consultation with the responsible Editor concerned.

(c) In conjunction with the responsible editors of the two journals and with the Director of The Wistar Institute, the outlining of the broad, general policies in the conduct of the journals.

(d) The making of an annual report to the Association concerning journal policies.

The report of the Committee was formally adopted by the Association.

The Executive Committee in conformance with Section 2 of the report later nominated ten members of the Association as candidates for membership on the Journal Committee of five. One other name was added by nomination from the floor.

Members of the Association then cast their ballots for five of these names with the following result:

C. R. STOCKARD was elected to serve for five years;
G. L. STREETER to serve for four years;
C. M. JACKSON to serve for three years;
C. R. BARDEEN to serve for two years; and
F. T. LEWIS to serve for one year.

The terms of service were arranged according to Sec. 2 of the report.

A proposed change in the constitution affecting the length of term for the several officers of the Association was voted upon and defeated.

The president announced the nomination by the Executive Committee of Charles R. Stockard as the representative of the Association in the Division of Medical Sciences of the National Research Council.

On motion the nomination was accepted and the nominee elected to represent the Association.

The business session then adjourned.

SATURDAY, MARCH 26. A SHORT BUSINESS SESSION FOLLOWED THE MORNING SCIENTIFIC SESSION.

The President announced that he had appointed Professor Ross G. Harrison as a delegate to represent the Association at The Second International Eugenics Congress which meets in New York City, September 22-28, 1921.

The Journal Committee reported the selection of Charles R. Stockard as Managing Editor of *The American Journal of Anatomy*, and John Lewis Bremer as Managing Editor of *The Anatomical Record*.

The place on the Journal Committee made vacant by the selection of Dr. Stockard as a Managing Editor was filled until the next annual meeting by the appointment of Dr. Ross G. Harrison.

President McClure was requested to present the greetings and best wishes of the Association to Professor George A. Piersol who was ill at his home in Philadelphia and unable to attend the meetings.

Professor S. W. Ranson introduced the following resolution:

RESOLVED: That the Association express its sincere thanks and appreciation to The Wistar Institute of Anatomy and Biology and to the local committee for the exceptional facilities and accommodations which have served to make the meeting a marked success, and for the cordial hospitality that has been so generously extended to all in attendance.

Unanimously voted.

On motion the Session adjourned.

CHARLES R. STOCKARD,

Secretary of the Thirty-Seventh Session of the
American Association of Anatomists

ABSTRACTS

1. *On the development of the ameloblasts of the molars of the albino rat, with special reference to the enamel-free areas.* WILLIAM H. F. ADDISON and J. L. APPLETON, Jr., University of Pennsylvania.

The crowns of the molar teeth in the albino rat, as in other rodents, have enamel-free areas on the cusps. These areas are always destitute of enamel from the time of first formation of the crown. The development of the enamel organ in these teeth is interesting, because of the differences which the functional and non-functional ameloblasts exhibit at different stages. The structure of the young enamel organ is similar to that of ordinary mammalian teeth. Up to the time of first formation of enamel and dentine (seen at first day after birth in first molar), all the cells of the ameloblastic layer are similar in size and structure. Soon after enamel formation has begun, however, differences appear in the formative and non-formative ameloblasts. Both classes of cells continue to grow for a time, but the non-formative cells grow more slowly and never attain the height of the formative cells. By the time the formative cells have attained their greatest height, the non-formative cells have begun to diminish in size. This diminution in size continues until the tooth erupts. At sixteen days the functional ameloblasts of the first molar measure 21μ and over in length and the non-formative cells about 7μ . The developmental history of the enamel organ shows that this condition of enamel-free areas is secondary to the condition where enamel covers the entire crown. This again is evidence that persistently growing teeth (in which enamel is always to some degree lacking) have been derived from rooted teeth.

2. *The oestrous cycle in the mouse.* EDGAR ALLEN (introduced by R. J. Terry), Washington University School of Medicine.

Using Stockard and Papanicolaou's method of diagnosing oestrus by the cell contents of the vaginal fluid, I have studied the cycle in the mouse. The changes are similar to those in rats reported by Long-Evans ('20). The average duration of the cycle is from four to six days. External signs are a poor criterion of 'heat,' occurring in less than 60 per cent of cases, where oestrus was shown to be present by cell changes. During oestrus there is little uterine discharge, the changes in the vaginal contents being due primarily to an alternate infiltration into, and absence of leucocytes from, the vaginal epithelium, and a periodic formation and destruction of the granular and horny layers. There is no bleeding into the lumen of the uterine cornua, nor any extravasation of red blood corpuscles into the stroma, but only a slight destruction of the mucosa by leucocytosis. There is a hypersecretion of the uterine glands during oestrus resulting in distention of the cornua, effected by a constriction of the cervix; the vagina being usually dry. Goblet cells are abundant in the epithelium of the oviducts. In some mice ovulation is spontaneous at every oestrus, so that

the ovaries are chiefly masses of corpora lutea. In others, where regular cycles have been recorded, no recent corpora lutea are present, while there are many atretic follicles which can be grouped to correspond to the recorded 'heat' periods. Consequently, all mice do not ovulate spontaneously during oestrus, and some ovulate only sporadically.

3. *Ovogenesis in the sexually mature mouse.* EDGAR ALLEN (introduced by R. J. Terry), Washington University School of Medicine.

The question of the formation of definitive ova (those ovulated during sexual maturity) is still an open one. According to different investigators, they have been derived from, (1) the primordial ova; (2) from an embryonic proliferation of the germinal epithelium; (3) from a similar proliferation between birth and sexual maturity, and, (4) in a few instances by the continuance of ovogenesis from the germinal epithelium during the sex life of the individual. Kingery derives the definitive ova, in the mouse, from the germinal epithelium during a period from three to forty days after birth, stating that it does not continue after that time. At sexual maturity cyclic changes appear in the genital organs. The period preceding oestrous is the period of augmented growth. In ovaries of several mice killed at this time I have found a complete series of stages in ovogenesis from the germinal epithelium identical to those figures for earlier stages by Kingery. Therefore, ovogenesis is not complete at birth or before puberty, but continues on into sexually mature life, and the germinal epithelium of the ovary is homologous to that of the testis tubules.

4. *On monozygotic human twins.* LESLIE B. AREY. Northwestern University Medical School.

Two specimens of early monozygotic human twins, each case unique of its kind, are presented. The first comprises twin embryos, each 12.3 mm. long, contained within a single amnion and chorion; except for some shrinkage of the entire specimen, the embryos are normal. Each possesses its own umbilical cord and yolk-stalk; the latter are inserted separately on a common yolk-sac. This furnishes for the first time direct proof of the origin of human identical twins from a single ovum. The second specimen is of normal monozygotic twin embryos, each lying within its own amnion. One member of the pair (11.5 mm. in length) has a normal yolk-stalk and sac (4.5 x 6 mm.); the other individual (12 mm. long) lacks these structures completely, as gross and microscopic examination prove. Certain inferences are suggested: 1) Human monozygotic twins do not result from the separation of blastomeres or blastomere clusters at the earliest stages of cleavage, but from a later fission of the inner cell mass. 2) Nevertheless, the human ovum appears to be rather rigid or determinate in its development; at least, in this case one embryo received all the yolk-sac formative cells. 3) The yolk-sac is not necessary for growth or differentiation; in fact, the twin individual lacking a yolk-sac is slightly the larger, while the correlation of menstrual age and body size coincides with the norm. 4) The yolk-sac and -stalk are not prerequisite to vasculogenesis; here was performed, as perfectly as ever may be expected, a natural experiment of ablation which demonstrates the independence of the embryo from such angioblastic ingrowths.

5. *The motor cortex of the brain of the sheep.* CHARLES BAGLEY, JR., Psychiatric Clinic, Johns Hopkins University.

A demonstration covering the histological study of the cortex of the brain of the sheep was given at the 1916 session of the American Association of Anatomists. The present communication is limited to the motor area of the brain of the sheep.

The motor cortex, as outlined in the early studies on the basis of histological structure alone, has been studied through means of electrical stimulation and some important differences brought out. The chief difference is the extension forward of the motor area to the most anterior pole of the brain and the elimination of an area of large pyramidal cells posterolateral to the principal motor area in the superior frontal convolution. Six areas can be satisfactorily outlined, the first three in the superior frontal convolution. Stimulation of the first two areas in the posterior extremity of the gyrus produces response in the limbs of the same and the opposite sides, while stimulation of the third area gives conjugate movement of the head and eyes to the opposite side. Area 4 lies between the olfactory sulcus and the outer prolongation of the coronal sulcus, and when stimulated gives contraction of the face muscles, more marked in the lower lip of the opposite side. Area 5 is just to the outer side of the coronal sulcus in the mesial portion of the middle frontal convolution; stimulation of this area gives response in the face muscles of the same side. The cortex giving response to electrical stimulation has been extirpated in three parts and the material stained by the Marchi method. The first extirpation area was the entire superior frontal convolution and included areas 1, 2, and 3. The second extirpation area included stimulation area 4, namely, that for the control of the opposite face muscles, while the third included area 5. Degeneration is clearly demonstrated in the fibers of the pyramidal tract in all of the extirpation specimens; these fibers cannot be traced beyond the upper cervical cord.

6. *The morphologic index.* R. BENNETT BEAN, University of Virginia.

A new index has been devised whereby any measurable character of a race, a group, a type, or an individual may be represented by a single numerical symbol. This symbol is plus or minus, depending upon whether it is above or below the world average of the character. The morphologic index is actually the percentage above or below the average. This may be illustrated by contrasting a few morphologic indices of the Scotch and Negro.

Morphologic indices

CHARACTER	SCOTCH	APPROXIMATE WORLD AVER- AGE	NEGRO
		<i>cm.</i>	
Stature	+10.30	165	-6.07
Nasal index	-18.75	80	+18.75
Cephalic index	-2.50	80	+3.75
Skeletal index	+0.96	52	-2.88

The nasal index differentiates the Scotch and Negritos more than do the other three factors, and the stature is the next best differentiator.

The actual stature may be obtained from the morphologic index by multiplying the world average by the morphologic index and adding the result to or subtracting it from the world average. The actual stature of the Scotch is 175 cm. and of the Negritos is 148 cm. The nasal index, cephalic index, etc., may be obtained in like manner.

We may take the morphologic index of any group of Scotchmen or Negritos, or of any type within the group, or of any individual, and compare them in many ways.

The morphologic index gives a numerical symbol that is simple, exact and convenient. It enables one to see at a glance the extent of variation from the world average, and thus to evaluate any factor, to determine its usefulness as a differentiator of race, group, type, or individual. It may also obviate the use of such terms as dolichocephalic, mesocephalic, brachycephalic; leptorrhine, mesorrhine, platyrrhine; leptoprosopic, mesoprosopic, euryprosopic; macroskele, mesatiskele, brachyskele.

7. *The value of sections of the body in teaching surgical and medical anatomy.*

CHARLES W. BONNEY, Jefferson Medical College.

The object of this paper is not to describe the preparation of the sections of the body nor to discuss their value in teaching descriptive anatomy. The former is thoroughly understood by modern anatomists, the latter in use in numerous American Medical Colleges. It is desired to emphasize the value of sections in teaching applied, surgical and medical anatomy. For that purpose they have been employed at the Daniel Baugh Institute of Anatomy of the Jefferson Medical College for the last seven years. A brief description of the methods used together with illustrative examples will be presented. Lantern slides will be used.

8. *The middle period in the development of the cloaca in chick embryos.* EDWARD

A. BOYDEN, Harvard Medical School.

This abstract deals with a portion of a comprehensive study embracing the development of the hindgut and associated regions in four species of bird embryos. Attention is called at this time to only a few points of interest: to the expansion of the allantois within the body cavity to form a pars coelomica of that organ; to the formation of a temporary urodaeal sinus which resembles in a striking way the adult urodaeal chamber of certain snakes and lizards which functions in these animals as a dorsal bladder; and to some new facts concerning the origin and nature of the bursa of Fabricius.

Up to this time the primordium of the bursa has been usually described as a swelling in the posterior dorsal wall of the cloaca caused by the coalescence of vacuoles arising within the cloacal membrane during the fifth and sixth days of incubation (cf. Lillie, p. 317). This description gives the bursa a unique origin, setting it apart from all other derivatives of the gut tract and adding one more difficulty to the interpretation of an organ which has been a bone of contention among anatomists since its discovery in 1604 by Fabricius, who ascribed to it the function of a receptaculum seminis. As a result of a quantitative study of

chick embryos between the fourth and fifth days of incubation, designed originally to explain the nature of accessory diverticula found between the rectum and anal plate, it has been possible to demonstrate that the primordium of the bursa appears a day earlier than hithert supposed and in the form of a caudally directed diverticulum whose cavity at first is in direct continuity with the cavity of the cloaca. In its later development the bursa unites with the protodaeum in a manner that closely resembles the union of the proctodaeum with the anal sacs in turtle embryos. And there are other resemblances between these two organs which superficially suggest an homology. Whether this proves to be true or not it is probable that the bursa should be classed with those derivatives of the gut tract, likewise arising as diverticula, whose functions are now obscure, but whose histogenesis suggests lymphoid degeneration.

9. *The early morphogenesis of the cerebral hemispheres of Amblystoma.* H. S. BURR, Yale University, School of Medicine.

A study of the early morphology of the cerebral hemispheres was suggested by some interesting results obtained in an experimental study of regeneration in the forebrain of *Amblystoma*. Evagination of the lateral wall of the neural tube occurs in the region of the confluence of the *S. limitans* and the *S. diencephalicus ventralis* and involves that portion of the lateral wall which intervenes between it and the lamina terminalis anteriorly. In this region lies the anlage of the olfactory bulb and the adjacent secondary olfactory centers, the latter crossed by the *S. diencephalicus medius*. The point of most rapid growth lies at the anterior end of the *S. ventralis* and seems to involve a short portion of the neural tube which lies between it and the lamina terminalis. Relatively little of the wall of the forebrain is evaginated, the definitive hemisphere growing very largely through the rapid increase in the number of cells forming the outpouching. This growth occurs principally at the anterior pole, producing rapid anterior elongation of the hemisphere. Growth in the dorsal and posterior region is much slower though greater than in the ventral region where growth is largely produced through the thickening of the walls. The successive development of fiber-tract systems shows that many nuclei develop in the hemispheres as a result of the ingrowths of the fiber tracts into the region involved. The nucleus medianus septi particularly shows evidence of growth after the appearance of the portion of the median forebrain tract which runs to it. From previous experimental work it can be stated that the primordia of the gray nuclei will develop to some extent without the ingrowth of tract systems, but the complete size development occurs only after nervous connections are established.

10. *The growth of the external dimensions of the human body in the fetal period and its expression by empirical formulae.* (Lantern.) L. A. CALKINS (introduced by R. E. Seammon), Department of Obstetrics and Gynaecology, University of Minnesota.

A graphic and mathematical analysis of measurements of seventy external dimensions of the body of upward of 400 preserved fetuses 2.3 to 54 cm. in length. The uncorrected curves of these dimensions (plotted against body length) are of three types: *a*) straight lines; *b*) curves approaching straight lines, but deflected upward toward their terminations; *c*) curves approaching straight lines but

deflected downward toward their terminations. Many larger specimens were injected. An extensive study of this technique shows that this causes the upward trend in most *b* curves, and that when eliminated they become straight lines. All downward deflected curves are of head dimensions affected by birth-molding. Observations on comparatively unmolded heads (breech extractions and caesarian sections, prove that these also are really straight. Only five curves (median-line measurements of upper parts of the body probably affected by head flexion) are not straight after elimination of artifacts.

External bodily dimensions plotted against body length (being, in general, straight lines) are expressed by the empirical formula, $Y = aX \pm b$ (*Y*, dimension; *X*, body length; *a* and *b*, constants). The constant *b* is positive for the head, zero for the thorax, and negative for the abdomen, pelvis and extremities.

It may be concluded: 1) The relative growth rates of the external body dimensions are established in the third month and remain unchanged until birth. 2) The growth of the external body dimensions in the fetal period follows the law of developmental direction.

11. Studies on the dynamics of histogenesis. IV. The biomechanical interaction of differential growth as a factor in the origin of bone. EBEN J. CAREY, Marquette School of Medicine.

Increased density or condensation is the chief physical property which characterizes osseous tissue. Is this quality self-engendered in the tissue involved or is it the mechanical resultant of the interaction of differential growth? In a former communication by the writer evidence was presented in support of the idea that embryonic bone is the immediate consequence of induced stresses and not the product of an anticipated function. Many workers on bone development consider that stresses are induced in, and strains manifested by the skeleton only after birth when the body weight is sustained. If such is the case, why does bone form in the upper extremity of man at all? Experiments which have been devised to disprove the mechanical origin of bone have not carried their point. The fact that the blastemcartilaginous skeleton is an area of accelerated longitudinal growth and that the surrounding soft parts are retarded in longitudinal growth has been entirely overlooked.

Two areas in synectial continuity and manifesting differential growth, as the skeletal and soft areas in the limb, exert an interaction. The zone of accelerated growth drags along the one of retarded growth, the latter in turn tends to slow down the speed or deflect the course of the former. This active interplay between growing parts tends to a dynamic equilibrium, but as long as one growing part is dominant and the other subdominant, growth and the resultant interaction and differentiation continue. The effect of interaction in the experimental production of double monsters is excellently treated in a recent monograph by Stockard (*Am. Jour. Anat.*, 1921, vol. 28, pp. 115-277).

The stresses induced in the origin of bone are the result of growth and resistances. The accelerated growing blastemochondrogenous skeleton meets the following resistances: 1) Opposed growth of contiguous skeletal segments; 2) weight of related soft parts; 3) reactive elasticity of traction of the soft parts retarded in growth; 4) active muscular pull. It is imperative, therefore, that, 1) *Growth* and 2) *Resistances* to growth be understood by the embryologist before

he can appreciate the importance of each factor. Both are active and formative during development, both are absolutely necessary to the realization of form and neither processes can be looked upon as more important in development than the other.

12. V. *The law of density of a growing tissue: On the progressive augmentation of femoral density as the resistances to the growth of the femur increase.* EBEN J. CAREY, Marquette School of Medicine.

With the rapid increase of limb weight, and with increase of opposition to growth at the ends of the femur, together with the resistances manifested by muscular reaction, the density of the femur increases progressively. This increase in density is concomitant with the relative decrease in femoral volume as the growth of the limb advances. In an 18-mm. embryo the volume of the femur constitutes one-third of the entire limb, whereas its density is 0.33. In a 20-mm. embryo femoral volume is one-fourth that of the limb and its density is 0.37, whereas in the 50-mm. embryo the volume of the femur is one-seventh and the density is 0.43. The density of the femur in a 200-mm. embryo is 1.6 and the volume is one-sixteenth that of the limb.

THE LAW OF DENSITY OF A GROWING TISSUE: *The density of a growing tissue is directly proportional to the resistances (pressure) encountered during growth.*

13. VI. *The law of relative volume of a growing tissue: On the relative decrease of femoral volume as the resistances to the growth of the femur increase.* EBEN J. CAREY, Marquette School of Medicine.

The various steps in the increase of skeletal density, from the blastemal to the cartilage period, and, secondly, from the cartilage to the osseous period, in skeletal condensation, are considered simultaneously with those changes, extrinsic to the zone of femoral formation. During the early stages of development, the weight of the entire hind limb is supported by the femur's acting like a cantilever beam. The weight of the limb increases rapidly. In an 18-mm. pig embryo the femur constitutes one-third of the volume of the limb and supports a weight of 0.013 grams. In a 20-mm. embryo the femur constitutes one-fourth the volume of the limb and supports a weight of 0.018 gram, whereas at the 50-mm. stage of the developing embryo, the femur constitutes one-seventh of the volume of the limb and supports a weight of 0.25 gram. Later at the 20-cm. stage the femur constitutes only one-sixteenth of the volume of the limb, but it supports the greatly increased weight of 30 grams. In addition to sustaining the above weight, the femur is opposed in growth by the accelerated growth centers located proximally and distally. Finally, as development continues, the resistance presented to longitudinal femoral growth by the contracting musculature and elastically reacting soft parts are opposing factors to be considered as extrinsic pressure limiting the relative volume of the femur to the thigh, as growth continues.

THE LAW OF RELATIVE VOLUME OF A GROWING TISSUE: *The relative volume of a growing tissue is inversely as the resistances (pressure) which it bears.*

14. VII. *On the torsion of the developing femur.* EBEN J. CAREY, Marquette School of Medicine.

That the femur undergoes a torsion during development has not been previously observed. This twist is objectively evident by observing the ventral aspect of a closely graded series of developing femora from the time the femur is approximately 3 mm. in length until it is 30 mm. in length. In a 3-mm. femur the head is in a direct line with a plane projected through the mid-ventrodorsal aspect of the shaft cutting through the center of the articular surface for the patella. This is objectively evident in a 3-mm. femur. With the next marked advance in development in a 9-mm. femur we find the head displaced mesiad. This torsion of the femur through an arc of 90° is in reality due to the torsion and development of the greater trochanter influenced by the traction of the attached gluteal muscles. This twist of the femur corresponds in time with the beginning and ending of limb rotation and with the period of greatest growth, differentiation, and activity of the thigh musculature.

15. VIII. *The law of joint formation: Bio-mechanical interaction of differential growth as a factor in the origin of joints.* EBEN J. CAREY, Marquette School of Medicine.

The blastemal skeleton of the acetabulum and the femur is apparently continuous. The femur, tibia, and fibula, and the foot plate progressively appear in the order named by the direct extension of the accelerated proliferation of the blastemal skeleton, comparable to the progressive caudal formation of metameres in the chick embryo. The first radical change from the apparently continuous to the segmental skeleton is seen in an embryo, 16 mm. long, by the appearance of a faintly curved line of compressed nuclei in the region of the future hip-joint. In an embryo 18 mm. in length another compression line is detected in the region of the future knee-joint.

1. By the continued opposition to growth between the contiguous centers of the segmental blastemal skeleton, mechanical compression occurs revealing the location of the future joint cavities.

2. The contour of the opposed surfaces constituting a joint is dependent on the intensity of the force of growth, per square millimeter of cross-section, of growing segments opposed in action, together with the force of muscular pull. That segment will possess the ball of a ball-and-socket joint which possesses the greater force of interstitial growth, longitudinally per square millimeter of cross-section.

3. Joints, therefore, are not the cause of skeletal segmentation, they themselves are the mechanical resultants of compression of prior centers of accelerated growth, opposing each other in action in the segmental blastemochondrogenous skeleton.

4. THE LAW OF JOINT FORMATION: *The contour of the opposed surfaces forming a joint is dependent upon the intensity of the force of interstitial growth, per square millimeter of cross-section, of the segments forming a joint and upon the resistances to the growth of each skeletal segment.*

16. IX. *The law of direction of myogenesis: The bio-mechanical interaction of differential growth as a factor in the origin of muscular tissue.* EBEN J. CAREY, Marquette School of Medicine.

Is the physical property which characterizes the initiation of muscular differentiation, namely, specific elongation of the nuclei and spongioplasm, caused by a factor intrinsic or extrinsic to the zone of myogenesis? The writer has presented evidence of direct observation which proves that the latter and not the former is the case. In other words, muscle is the resultant of the tension, pulling out or traction to which a syncytial mass of mesenchyme is intermittently but progressively subjected by a related region of cells accelerated in growth.

It was shown formerly that the dominant zone of accelerated growth in the intestine is the epithelial tube. By expansion of the epithelial tube in spiral growth the surrounding mesenchyme was drawn out in tension resulting in heliocolid muscular differentiation. In the limb the zone of accelerated growth is the central segmental skeletal core. This draws out by traction the surrounding mesenchyme resulting in skeletal muscular differentiation. The zone of accelerated growth in the cardiac area is the progressive increase in the whirling volume of the blood. This draws out the surrounding mesenchyme in tension corresponding to the direction of the vortex of blood resulting in spiral muscular differentiation. The detail proofs for these assertions will soon be published.

THE LAW OF DIRECTION OF MYOGENESIS: *The elongation of a developing muscle is in the direction of the accelerated growth of an extrinsic dominant zone which draws out in tension the mesenchyme forming the muscle.*

17. *The development of the aster in the artificial parthenogenesis of the sand-dollar egg.* ROBERT CHAMBERS, Cornell University Medical College.

No noticeable changes occur in the cytoplasm or the nucleus of the eggs until long (half an hour or more) after both the butyric-acid and the hypertonic-solution treatments. The visible phenomenon peculiar to the parthenogenetically induced egg consists in the manner in which a fluid substance begins to separate out of the egg cytoplasm, preparatory to the formation of the preliminary single aster. In the sperm-fertilized egg radiations appear immediately about the sperm head, and the accumulation of the fluid substance is from the very start through the agency of the ray-like channels of the growing aster.

An optimum parthenogenetic treatment causes vacuoles to appear which fuse to form a central clear area about which radiations develop until an aster is formed corresponding exactly with the fully developed sperm aster of a normally inseminated egg. From now on the procedure is similar to that occurring in a sperm fertilized egg.

Over-treatment causes the appearance of many vacuoles scattered throughout the egg resulting in multiple asters. Under-treatment may result in the formation of a single aster which, however, periodically disappears and reappears as a single aster. A successful treatment not only causes a separating out of a liquid from the egg cytoplasm, but also induces a polarity within the resulting clear area to enable it to form two centers about which an amphiaster may develop.

18. *The reaction of living cells in the tad-pole's tail toward injected starch granules.*

ELIOT R. CLARK and ELEANOR LINTON CLARK, University of Missouri.

Small quantities of starch (corn-starch and arrow root) were injected into the transparent tails of frog larvae and the region of injection studied in the living during the subsequent hours and days.

Uncooked starch, boiled starch, and starch cooked just to the point of gelatinization were tried. The larvae were fixed in iodine at different periods of time after the injection.

Toward the uncooked starch granules the response was similar to that displayed toward foreign bodies, such as carbon and carmine. Leucocytes approached the starch grains and engulfed them and the starch remained inside the leucocytes indefinitely (over a month).

The boiled starch grains disintegrated within the first half hour after injection and after an hour no stain was obtained after treatment with iodine. Wandering cells moved toward the injection site.

In the case of the semi-cooked starch, near-by wandering cells moved very rapidly toward the injected material and within twenty minutes leucocytes began to emigrate from neighboring blood-vessels in very large numbers. Within an hour the starch grains were all inside of leucocytes. The diapedesis of leucocytes continued for six hours or more. Leucocytes staining blue with iodine were demonstrated from three to four hours after the injection. The further stages in digestion were not followed since the characteristic reaction of dextrin or of glycogen was not obtained and our microchemical tests for sugar, injected into the tail fins, were unsuccessful.

Starch cooked to the point of gelatinization proved to be a most powerful chemotactic agent for leucocytes. The other tissue cells showed no response toward injected starch.

19. *Cyclic changes in the ovaries and uterus of the sow, and their relation to the mechanism of implantation of the embryos.* GEORGE W. CORNER, Johns Hopkins Medical School.

The author has completed a detailed study of the follicles, corpora lutea, and uteri of a large series of pregnant and nonpregnant animals killed at known stages throughout the oestrous cycle. The cycle averages twenty-one days in length. Ovulation is found to occur during oestrus; the corpora lutea complete their formation about the seventh day, and remain in full development from the seventh to the fifteenth day, thus surviving just long enough to cover the period of attachment of the embryos. If no embryos are present the corpora lutea degenerate about the fifteenth day.

A few days before and during oestrus the uterine epithelium is in a state similar to that described by Stockard and Papanicolaou and by Long and Evans in the small rodents; but during the growth period of the corpus luteum the uterus undergoes histological changes culminating, from the eighth to the tenth day, in a state of enhanced epithelial activity. At this time the embryos, when present, are still unattached and are being shifted into position for implantation. From the tenth to the fifteenth day (the period of implantation), further elaborate changes take place by which the epithelium is brought to a state characteristic of early pregnancy in the implantation stage. If no embryos are present the same changes occur, but subside after the fifteenth day.

These results indicate that there is a correlation between the state of the corpus luteum and that of the uterus by which the uterus is prepared, after each ovulation, to receive embryos. A detailed and illustrated monograph will appear in the publication of the Carnegie Institution.

20. *Digestion of different proteins by the mesenchyme and its derivatives in the tadpole.* (Lantern.) VERA DANCHAKOFF, Columbia University.

Though well known to exist within the multicellular organism the phagocytic digestive activity of the mesenchyme has not been much studied. Little is as yet known regarding the amount of digestive work accomplished in the organism by the mesenchyme, if given opportunity. Neither is the extent known to which this activity becomes a factor in the resistance which a multicellular organism offers to the growth of heterogeneous tissues even of such a great proliferative capacity, as, for example, the tumors.

The adult splenic mesenchyme of the fowl, as shown by me last year, is capable of ingesting and digesting, one by one, cells of a mammalian proliferating tumor. The mesenchyme and its derivatives, in the form of small wandering cells, in various tadpoles, will be shown to possess the power of digesting various proteins. The mesenchymal tissue within the tail of different tadpoles can be fed on finely particulated fibrin, edestin, coagulated albumen, and lecithin, the particles being of the size of a small fraction to a few diameters of a mesenchymal cell. The response to the sudden appearance of a large quantity of injected material is rapid from the part of the mesenchymal and wandering cells. Four to six hours after injection a great number of mesenchymal cells and of cells of the small lymphocyte type are found around and within the injected mass; about twenty-four hours after the injection all but the largest particles are ingested, and after three to four days no trace of the injected material is found.

The results of these experiments illustrate the great digestive capacity inherent in the mesenchymal and small lymphocyte cells of the amphibia during the tadpole stage. These cells can most effectively take care of comparatively huge masses of injected particulated protein, and like physiologically balanced unicellular organisms, if given opportunity, successfully perform their own digestive activity.

21. *Further morphological evidence for the digestive capacity of adult splenic mesenchyme in the fowl.* VERA DANCHAKOFF and S. M. SEIDLIN, Columbia University.

A new morphological evidence for the digestive capacity of the mesenchyme was brought forward by Danchakoff last year. The splenic reticular cells of the adult fowl were shown to be capable of surrounding and digesting the living cells of an actively proliferating mammalian tumor (the Ehrlich sarcoma). The encircling of the tumor cells by the mesenchyme, followed by digestion, as observed in Danchakoff's experiments, is the result of the immediate encounter of two living tissues. A further study of the digestive capacity of the mesenchyme was required in order to ascertain whether the living tumor cells were treated by the mesenchymal cells in the same manner as dead particles of mammalian protein would. Small particles of catgut were intimately mixed with mash of adult splenic tissue and grafts of this tissue made on the allantois of seven days chick embryos.

The study of the grafts after five days' growth has shown the particles of catgut partly digested, partly attacked by the mesenchyme. Mesenchymal cells isolated and in the form of more or less huge plasmodia surround the catgut particles, the latter showing distinct indentations, which in outline often correspond to mesenchymal cells. The mesenchymal plasmodia close to the partly digested catgut contain vacuoles. The process of digestion where the catgut particles are small is very similar to that exercised by the mesenchyme against the cells of the Ehrlich sarcoma. The splenic mesenchymal cells of the adult fowl seem to be capable of exercising phagocytic and digestive activity regardless of whether this activity is directed against sterilized particles of heterogeneous dead tissue or against living heterogeneous tumor cells of certain physicochemical constitution.

22. *A new interpretation of the morphology of the nervous system.* RAYMOND A. DART and JOSEPH L. SHELLSHEAR (introduced by R. J. Terry), University of London.

His ('68) promulgated his principle of ectodermal origin of neural tissue. Balfour and others extended this hypothesis to postulate a neural tube origin of all neuroblasts. Beard, Platt, Landacre, and others have shown that a large proportion of the cranial ganglionic elements arises in the ectoderm lateral to the medullary area from certain areas called 'placodes.' Observations by J. P. Hill, Elliot Smith, and the authors have demonstrated a similar peripheral but entodermal origin in placodes for the visceral elements in the VII, IX, and X cranial nerves throughout Vertebrata. A radical revision of current conceptions is therefore necessitated.

The 'placodal' principle of a peripheral origin for all neuroblasts of the peripheral nervous system is of general application. The only point of agreement between students of the ontogeny and phylogeny of the sympathetic system is of the first appearance of the so-called 'primary anlagen' peripherally and in inextricable relationship with the mesodermal structures supplied thereby. That the sympathetic system develops independently of the neural tube was shown by Weber in 1851. A mesodermal origin of these neuroblasts must therefore be postulated and is demonstrable.

But these are not the only mesodermal neuroblasts. Concurrently with the differentiation of the somite from indifferent cells into the various 'supporting tissues' of the body there arise from similar 'indifferent cells' of the primitive somite the neuroblasts for the innervation of these tissues. The somite, then, has this property in common with definitive placodes previously described by various authors; it gives rise to a) neuroblasts and b) supporting tissue. A rational phylogenetic and ontogenetic explanation is provided in this way for the proprioceptive senses. The anterior horn cells of the neural tube must, however, be appreciated as primarily 'extraneural.' The neural tube itself is considered as a series of bilaterally segmented placodes. The data entail further a revision of the conception of neurobiotaxis as put forward by Ariens Kappers. This principle is given wider application for the interpretation of the movements of sensory neuroblasts which move away from the 'source of stimulus.' The nervous systems of Vertebrata and Invertebrata are harmonized by the 'placodal' conception, and an hypothesis is promulgated to account for the origin of the

former. Finally, the problems of segmentation and the mesoderm are deemed to be more correctly appreciated from the new point of view.

23. *Degeneration phenomena in the pelvic gland of the male Necturus.* A. B. DAWSON, Loyola University School of Medicine.

Pelvic glands of males, killed during the late autumn and winter, exhibit degeneration phenomena similar in many respects to those recently described by Saguchi ('20) in the frog's pancreas under the title, 'physiological degeneration.' Although numerous cells are degenerating, the gland is secreting actively. Nucleoli are not demonstrable in the normal cells and it seems impossible to interpret the large eosinophilic central corpuscle of the 'chromocyte' as being a result of nucleolar hyperchromasy. The nucleus undergoes successive changes characteristic of Flemming's chromatolysis. Some degenerating cells escape directly into the lumen of the tubule; others, however, are sooner or later taken up by neighboring normal secreting cells. Within the normal cells the plasma of the degenerating cells is digested and absorbed rapidly. The degenerating nuclei usually become separated into several portions, either by direct fragmentation or a process of gemmation, and are ultimately eliminated, along with the secretion of the normal cell, into the lumen of the tubule. No phagocytosis was observed in connection with this degeneration and no mitosis was evident at this period of the year. These intracellular corpuscles, derived from degenerating gland cells, resemble 'nebenkerne' and have been so interpreted by many investigators working on glands. The small spherical nuclear fragments simulate basophilic secretion granules.

Pelvic glands from animals killed in July present a very different picture. The lumina of the tubules are reduced to a minimum and mitotic figures are encountered very frequently. Large phagocytes filled with disintegrated cells are numerous.

24. *The growth of the brain and the spinal cord in the human fetus and its expression by empirical formulae.* HALBERT L. DUNN (introduced by R. E. Scammon), University of Minnesota.

A quantitative study of the growth of the brain and its parts and of the spinal cord in a series of 156 human fetuses ranging from 4 to 56 cm. in crown-heel length. The growth of the central nervous system in the fetal period follows, in a general way, the growth of the body, and its increase in weight and volume may be expressed by formulae similar in type to that expressing growth in body weight. Further analysis shows that three distinct subdivisions or varieties of this general type of growth may be recognized in the central nervous system. These are, 1) cerebral growth, which shows a slow but steady increase prior to five and one-half or six months (ca. 30 cm. CH) and a constant and more rapid growth from that time to birth; 2) brain-stem and cord growth, which proceeds comparatively rapidly previous to the sixth fetal month and comparatively slowly thereafter, and, 3) cerebellar growth, which is characterized by a slow rate of growth prior to the seventh fetal month and by an extremely rapid rate of increase thereafter.

25. *Hematological and respiratory conditions in the larval stages of the lungless amphibians, Batrachoseps attenuates and Aneides lugubris.* V. E. EMMEL, University of California.

In attempting to correlate the remarkable occurrence of non-nucleated erythrocytes in *Batrachoseps attenuates* (*Am. Jour. Anat.*, vol. 16, p. 180; *Anat. Rec.*, vol. 18, p. 232) with physiological factors, a comparative study was undertaken between this animal and *Aneides lugubris*. Both amphibians are lungless, have similar environments, but differ widely hematologically. It became necessary to carry the investigation into the larval stages. We were fortunate in securing one set of eggs for each species. A number of larval animals were removed from the egg before hatching and blood preparations made. In the larval *Aneides* all erythrocytes were nucleated, but in the larval *Batrachoseps*, on the contrary, non-nucleated erythrocytes were almost as abundant as in the adult.

It thus becomes evident that whatever physiological factors may be responsible for the marked hematological differences in these two species, they must be already operative before hatching. The larval respiratory gill structures show striking differences. *Aneides* has a very broad, three-lobed, leaf-like gill membrane, permeated by a complex capillary network. *Batrachoseps* has a simple, slender, three-fingered gill structure, traversed by a single vascular loop for each finger-like process. In *Aneides* the blood corpuscles pass through the gill capillaries in a single file, whereas in *Batrachoseps* the blood is carried through each vascular loop as a column of corpuscles in the manner of a small arteriole. In contrast to *Aneides*, therefore, we have in the larval *Batrachoseps* a respiratory mechanism relatively deficient in capillary exposure of the blood. This condition is apparently compensated for by the increased oxidation efficiency of the thin non-nucleated erythrocytic discs, thus furnishing a phylogenetic precursor of the erythrocytic efficiency finally attained in mammals.

(Further studies on the physiology of reproduction include abstracts 26 to 34.)

26. *Proportion of ova producing full-term young in the rat.* JOSEPH A. LONG and HERBERT M. EVANS, University of California.

We are beginning to appreciate the widespread and customary occurrence of departures from perfect functioning of the mammalian reproductive apparatus—departures which reduce fertility. These may be due to fault with ovary, tube, or uterus. They are occurring continually. During the last three years we have recorded the number of young in 625 litters of the rat. The average lies between six and seven. During this period of time fifty animals were sacrificed within one day after ovulation and at least one oviduct and ovary cut serially. In all instances the eggs from this ovulation were encountered in the tube and were enumerated. An average was found of 4.8 eggs in each oviduct or 9.6 eggs per ovulation. Other material in which the eggs could not be enumerated with reliability, but in which the corpora lutea of a single ovulation could be counted, was studied. This showed that five corpora per ovary or ten per ovulation represented the average. The animals from which data were secured concerning the number of eggs or corpora were treated in every respect as to food, cage space, etc., identically with the animals in which the number of litter young was

recorded. They were also in many cases litter mates of such animals. Evidently, then, under these conditions nine or ten ova are represented by only six or seven offspring carried to term.

27. *On the production of the condition of 'pseudopregnancy' by infertile coitus or mechanical stimulation of the cervical canal in the rat.* JOSEPH A. LONG and HERBERT M. EVANS, University of California.

We have previously shown that the advent of the next oestrus is delayed when the rat is allowed to mate with a vasectomized male or when the cervical canal is stimulated by the momentary insertion of a small glass rod. This pause, which we have proved to result from delayed ovulation, may be due either to some sort of direct repression of follicular growth or to a continuance of life of the corpora lutea which in the case of cattle have apparently been shown to hold off follicular growth and oestrus. The corpora lutea in these cases come to resemble those of pregnancy. As we have previously explained, we are to understand this remarkable response as a contrivance to insure implantation. The fact that deciduoma are difficult to produce during normal oestrous cycles, but can be produced after cervical stimulation, is in strict harmony with the idea that changes are thereby provoked which facilitate implantation. We may suppose something has occurred to 'activate' the corpora lutea. The corpora are affected through humoral paths, since these phenomena all occur with the transplanted ovary. But nervous pathways are probably concerned in initiating the change, for the products of abrasion of the cervical mucosa do not themselves cause these changes (Freyer; see below). The designation 'pseudopregnancy' is justified on further grounds than because of the prolongation of life span of the corpora lutea. Most striking is a change in the character of the vaginal epithelial mucosa. In pregnancy the vaginal mucosa becomes a high stratified epithelium, but with its surface cells columnar instead of squamous in type. Furthermore there ensues a characteristic vacuolization of its middle cell layers, a phenomenon beginning about the tenth day of gestation and reaching its greatest expression on the sixteenth day. These changes are inaugurated in the vaginal mucosa ten or more days after mechanical stimulation of the cervical canal.

28. *On the cause of the effects produced by stimulation of the cervical canal in the rat.* M. G. FREYER (introduced by H. M. Evans), University of California.

The delay in the appearance of the next oestrus and the condition of so-called pseudopregnancy produced by mechanical stimulation of the cervical canal in the rat has been shown by Long and Evans to take place in animals in which ovarian transplantation has recently been carried out. We can, hence, not refer this effect to the nervous connections of the ovary itself. It seemed possible that the slight injury to the cervical epithelium might lead to hormonal products which when absorbed and reaching the circulation thus affect the ovary directly or indirectly by means of some other endocrine gland.

At the suggestion of Doctors Evans and Long, six careful experiments were carried out in order to test this point. Epithelial scrapings were made from the lower portions of the cervical canal of six animals which were in the pro-oestrous period or at the transition between pro-oestrus and oestrus. This material, obtained under aseptic precautions, was immediately injected into the perito-

neal cavity of six other animals which happened to be at the same stage of the oestrous cycle. The succeeding oestrous cycles in the recipient animals were of normal duration. None of the characteristic effects of cervical stimulation were obtained. It would hence appear that the initial part of this mechanism is actually mediated by nerve impulses which, however, produce humoral changes so that the corpora lutea of recently transplanted ovaries, which can only be reached by the blood stream, are in some way invigorated and continued in function.

29. *A characteristic histology of the vaginal mucosa during lactation.* JOSEPH A. LONG and HERBERT M. EVANS, University of California.

During lactation the vaginal smear in the rat resembles closely the picture found during the dioestrous interval of the normal oestrous cycle, i. e., it consists of polymorphonuclear leucocytes and a variable content of irregularly sized epithelial cells. Nevertheless, the histology of the vaginal mucosa at this time differs widely from that found in the dioestrous pause. We have previously established the fact that ovulation does not occur during lactation. Ovulation is always heralded by characteristic changes in the structure of the vaginal mucosa and also in the vaginal smear. In both pregnancy and lactation ovarian function is manifested by actively secreting corpora lutea which in turn may be viewed as repressing all follicular growth and activity. We have shown in the preceding section that a characteristic vaginal histology occurs throughout gestation. It is also a fact that during gestation the vaginal smear resembles that of the normal dioestrous pause. Similarly during lactation characteristic changes occur in the vaginal mucosal histology without changes in the smear. The epithelium in one respect resembles that found in pregnancy in that it possesses a surface layer of cylindrical cells. But the gravid vaginal mucosa is high, that of lactation low. While on the second day of suckling this mucous membrane may consist of four or five cell layers, by the fourth day more than three layers are seldom encountered, and on the sixteenth day, when lactation may be assumed to be at its height, most of the mucosa consists of but two cell layers, the superficial of which is constituted by cubical or low cylindrical elements. The strict dependence of this characteristic epithelium upon the performance of the mammary glands, which divert and limit ovarian function to the corpora lutea, is illustrated in the most striking way when the young are removed. Within forty-eight hours after removal of the young the low columnar mucosa of lactation gives place to a high, stratified squamous epithelium.

30. *On the production of deciduomata during lactation.* JOSEPH A. LONG and HERBERT M. EVANS, University of California.

Our preliminary experiments seemed to indicate that deciduoma were not easily produced by the contact of foreign bodies with the uterine mucosa during lactation. We were consequently under the impression that the rarity of conception during lactation might be referable to an unfavorable implantation reaction in some way associated with lactation. We have continued our operations upon the uterus during lactation. Typical deciduomata can be produced when the procedure is carried out at any time after the fourth day of lactation and the animal sacrificed one week after the operation. It is consequently neces-

sary to refer the well-known comparative immunity from a second gestation which characterizes the early period of lactation in all animals, to the lack of ovarian changes associated with both heat and ovulation, not to difficulties in implantation of ova. The existence of a vigorous deciduoma reaction during lactation when uterine atrophy normally occurs would appear to establish conclusively the relation of this response to the existence of functional corpora lutea, for in all conditions in which functional corpora are present the response can be elicited.

31. *Cyclic changes in the mammary gland of the rat associated with the oestrous cycle.* MONROE SUTTER (introduced by H. M. Evans), University of California.

The exact mechanism responsible for the assumption of function on the part of the mammary gland has received a considerable amount of attention during the last few years. Although an indirect nervous connection between mammae and uterus exists (influence of suckling on uterine contractions), it has been generally assumed that the development of the mammary apparatus is due to hormonal influences. As is well known, these hormones have been variously supposed to come from corpus luteum, placenta, or foetus. I have been encouraged by Doctors Evans and Long to study the changes which may be observed in the mammary glands of virgin female rats at various steps in the oestrous cycle. The study of sections was eventually abandoned and gross mounts were made of spreads of the entire glands which had been stained and cleared.

The following conditions have been detected: Toward the end of the pro-oestrous stage (stage 0 of Long and Evans) the mammary tree exhibits long, slender branches which have a few almost naked twigs projecting from them. Close inspection reveals that there are many minute bud-like processes on the twigs and on the main branches at infrequent intervals. In the next stage, oestrus (stage 1 of Long and Evans), when cornified epithelial cells are found in the vaginal smear, undoubted evidence occurs of pronounced growth in the mammary tree. The small buds on the mammary twigs have sprouted out to varying degrees and new ones have appeared. Instead of appearing generally smooth and naked, the branches and twigs are irregular and covered with numerous projecting buds. The size and shape of the branches vary greatly from branch to branch and in a given branch. This great variability appears to be one of the most marked characteristics of rapid growth. By the time leucocytes have appeared in the vaginal smear, evidences of further growth in the mammary tree can be seen in the increasing complexity of the secondary branches. By this time we know that ovulation has normally occurred and young corpora lutea have been formed. The end branchings of the mammary tree often form transparent bulb-like projections which vary greatly in shape and size. This complexity of the tree and some slower growth of it undoubtedly continue until near the next pro-oestrous stage when, possibly due to degeneration of the corpora lutea, regression occurs.

There is thus a regular cycle of growth changes imposed upon the mammary ducts, and although these are undoubtedly accelerated and are maintained by the corpora lutea, they may be detected and are quite marked before the corpora are formed.

32. *On the rapid maturation of the ovary by transplantation of the youthful gonad to adults.* JOSEPH A. LONG and HERBERT M. EVANS, University of California.

In order to determine whether we could produce an experimental precocity in the development of the remainder of the reproductive system, we attempted to transplant adult ovaries into young animals. As a matter of fact, an exchange of ovaries was carried out between immature animals from twenty to thirty days of age and adults between five and six months of age. The adult grafts succumbed, but in every instance the immature ovaries were vascularized and grew rapidly, although these also in some instances did not continue to function. It is, however, remarkable that in all instances at least one set of Graafian follicles and corpora lutea were produced by the infantile ovaries in adult hosts. Furthermore, these changes took place in from six to eight days and brought on typical oestrus of the adult host as seen by changes in the vaginal smear, behavior, etc.

It is apparent that endocrine influences of the adult tissues are responsible for provoking this sudden maturation of the sex gland, which normally occurs from one to two months later.

33. *The method of opening of the vagina in the rat.* K. O. HALDEMAN (introduced by H. M. Evans), University of California.

At birth the lumen of the vagina extends caudal to within 1.2 mm. of the external surface of the body. The structure closing the vagina consists of a solid, branching core of stratified squamous epithelium surrounded by compact connective tissue. This condition persists until, at about the age of thirty to forty days, several centers of cornification appear in this epithelial core and vesicles containing desquamated cornified material and leucocytes are formed. The vesicles enlarge and coalesce so as to form a lumen through the epithelial core. The first external sign of impending opening is a turgescence and wrinkling of the future lips of the vagina. Occasionally a median cord extends dorsoventrally across the vaginal orifice for several days after opening. Frequently a plug of cornified material protrudes from the opening soon after it is established. Sections through the vaginae prior to their opening in animals older than thirty days showed large masses of cornified material, non-cornified cells, and leucocytes in the lumen near its distal end. In some cases small areas of the vaginal mucosa were covered with cornified epithelium. Five cases were studied to determine whether or not ovulation had occurred, and in no instance was this a fact. These isolated patches of epithelial cornification must not be confused with the complete cornified transformation of the vaginal mucosa accompanying oestrus.

34. *On the association of continued cornification of the vaginal mucosa with the presence of large vesicles in the ovary and the absence of corpus formation.* HERBERT M. EVANS and JOSEPH A. LONG, University of California.

It has already been shown that in the normal oestrous cycle of the rat cornification changes in the vaginal mucosa are associated with the enlargement and maturation of follicles and that these changes cease at about the time of ovulation. Normally the cornified stage lasts about thirty hours.

As a rare anomaly (seven cases in about 800 rats) cornification of the vaginal mucosa may be greatly prolonged; instances of 2, 3, 5, 7, 11, 11, and even 21 days

have been observed. In one case in which cornification had persisted for five days and in two cases of eleven days the animals were tested and oestrus found to be still present. All of the seven rats were killed while this phenomenon was still in progress, and the ovaries examined in serial section. The most striking thing about them was the presence of large, fluid-filled vesicles, many of these possessing a thick, apparently healthy granulosa layer which together with the basement membrane is invaginated at many points by blood-vessels and containing what appeared to be normal eggs. Others were clearly undergoing degeneration, leading to the formation of large, thin-walled vesicles devoid of ova. In addition, the ovaries were notable by reason of the absence of normal healthy corpora lutea, those present being apparently in process of degeneration—quite markedly so in the one case of twenty-one days.

We have observed similar long cornified stages in the vaginal smears of two cases in which the ovaries were transplanted to the rectus muscle and in which also the ovarian findings resembled the above. This fact would appear to support convincingly the idea that these ovarian changes produce their effects on the vagina through humoral rather than nervous pathways.

(Experiments on the endocrine relations of the ovary in the rat include abstracts 35 to 38.)

35. *The effect of thyroid feeding on the oestrous cycle of the rat.* HERBERT M. EVANS and JOSEPH A. LONG, University of California.

Thyroid obtained daily from freshly slaughtered beeves was fed in doses varying from $\frac{1}{4}$ gram to an entire half gland. The rats used for feeding as also those for controls were selected from a large stock because they exhibited approximately regular four-day cycles.

In all cases thyroid feeding was accompanied by an increased consumption of food, but decrease in body weight. On the one hand, when the doses were larger ($\frac{1}{4}$ to $\frac{1}{2}$ gland) loss in body weight was pronounced and some animals succumbed, the cycle being greatly lengthened or inhibited altogether. On the other hand, when the doses varied from $\frac{1}{4}$ to $1\frac{1}{2}$ grams daily, amounts also sufficient to produce loss of weight with increased consumption of food, the oestrous cycles were usually not greatly disturbed. There consequently do not appear to be specific effects of thyroid substance on the oestrous cycle.

36. *The effect of thyroidectomy on the oestrous cycle of the rat.* HERBERT M. EVANS and JOSEPH A. LONG, University of California.

Both thyroids were removed from three groups of rats which lived a long post-operative life: thirty-one adults, seventeen young rats 37 to 54 days of age, and eleven suckling ones. In all cases there was no doubt but that by far the largest part of the thyroid was excised, and in the youngest rats the operation was performed under binocular microscopes. The mortality from the operation is low. In the case of the adults the operation was usually followed by a pause in the oestrous cycles of 6 to 27 days, but in turn succeeded by normal oestrous cycles, about twenty of which were observed.

The operations on both the young and suckling animals influenced appreciably neither the time of maturity nor the lengths of oestrous cycles. Several of the second group after reaching an age of several months were autopsied and

found to possess what appeared to be lobes of regenerated thyroid tissue in many cases almost of the size of normal glands. Sections found these to be thyroids which had regenerated in spite of the effort which had been taken to secure complete ablation.

37. *The effect of feeding the anterior lobe of the hypophysis on the oestrous cycle of the rat.* HERBERT M. EVANS and JOSEPH A. LONG, University of California.

Four sets of experiments were carried on, in two of which fresh glands were fed and in two dried hypophysis from Armour & Co. and from Parke-Davis & Co. In all cases the feeding was begun at weaning, on the twenty-first to twenty-third day, litter mates being used as controls. Daily observations were made to determine the opening of the vagina and cyclical changes in the vaginal smear. A total of fifty-five rats was used for the experiments and fifty-four for controls.

The anterior lobes were dissected from the glands of freshly slaughtered cattle and were ground, weighed, and fed within six hours of slaughtering. A half gram was given each rat, which had been isolated in a clean metal box where it could be seen that the total amount given was consumed, after which the animal was returned to its cage and given ordinary food. In one set the controls were not fed differently from their mates except for the hypophysis; but in the other set the controls were given in addition $\frac{1}{2}$ gram of raw liver daily to offset the possible nutritive value of the fresh hypophysis. Each rat was weighed at intervals of four days. In both sets the hypophysis-fed animals and controls showed neither significant differences in growth nor in the age of maturity and lengths of subsequent oestrous cycles. In the case of ten adult rats the total food intake was limited to 6 to 10 grams of the fresh anterior lobe and rolled barley. Their oestrous cycles (ten to twenty of which were observed) were not sensibly altered.

Similar tests were conducted with the dried commercial substance, except that no controls were given fresh liver. But for the fact that large doses could not be given without producing intestinal disturbances, the results were not substantially different from those given above.

38. *The effect of the anterior lobe administered intraperitoneally upon growth, maturity, and oestrous cycles of the rat.* HERBERT M. EVANS and JOSEPH A. LONG, University of California.

The anterior lobes were dissected from fresh glands, were immersed five minutes in 30 per cent alcohol, rinsed thoroughly in sterile Locke's solution, triturated with a small amount of sand, and centrifuged for about half an hour, care being taken to carry out all manipulations aseptically. The supernatant fluid from centrifuging was injected into the peritoneal cavity in amounts from $\frac{1}{2}$ to 1 cc., according to the age of the rat, the first dose being given at an age of about fourteen days. At the beginning a similarly obtained fluid substance from liver was given some controls, but soon discontinued because of its toxic effect. Every animal was weighed at intervals of five days, beginning with the twentieth day of age. To date daily observations have been carried to the eightieth day. The subjoined table shows a greater rate of growth of the experimental animals as compared with their controls, a disparity which is increasing.

At the same time the effect of the anterior lobe has been to repress sexual development by delaying sexual maturity and lengthening the oestrous cycles, in some cases oestrus being entirely inhibited.

In the case of five adult rats with previously regular four-day cycles, doses of 1 to 2 cc. of the anterior lobe fluid substance caused an immediate cessation of the four-day rhythm, the smaller doses permitting oestrus to recur at longer intervals, the larger inhibiting it altogether. These results are in marked contrast to the lack of effect produced by oral administration of the anterior hypophysis. As far as the influence on sex function is concerned, they are in marked contrast to prevalent opinion.

AGE	38 EXPERIMENTAL ANIMALS	38 LITTER MATE CONTROLS
<i>days</i>	<i>grams</i>	<i>grams</i>
14	20.2	19.02
20	31.6	33.14
25	48.6	46.2
30	61.7	60.0
35	80.6	70.7
40	95.6	86.2
45	117.6	109.0
50	140.8	126.1
55	159.5	139.25
60	177.0	153.3
65	197.2	165.6
70	214.5	173.7
75	227.8	183.5

39. *The digestion and assimilation of fatty food as determined by the aid of the dark-field microscope, and a fat-soluble dye (American sudan).* SIMON H. GAGE, Cornell University.

The findings on the above reported at the last meeting of the Association have been repeatedly verified on people of various ages and on animals of widely different species. That is, with a dark-field microscope one can determine by the number of particles (chylomicrons) present: *a*) whether the fat taken with the food is being digested and absorbed; *b*) the time required for the process; *c*) the comparative digestibility of a given fat by different individuals and different species of animals; *d*) the comparative digestibility of different fats by the same individual or animal.

In the further study of the subject it has been found not only possible to determine the appearance, increase, diminution, and disappearance of the fat particles (chylomicrons) in the blood or chyle by the dark-field microscope, but with the naked eye it has been easy to follow the digested fat from the intestines to the lacteals, to the lymph nodes and to the cisterna chyli, and through the thoracic duct to the blood-vessels, and in the blood-vessels to all parts of the body. This was made possible by the use of a fat-soluble dye (American sudan), which when once dissolved by the fat, sticks so tight to it that it never lets go through all the processes of digestion, although they may involve splitting the fats into fatty acids and glycerin or even the formation of soaps and their absorption and resynthesis by the intestinal epithelium. The color serves as a label, so to speak,

and enables one to follow it in all its wanderings, and to see where it is finally deposited when assimilated.

Contrary to the general assumption that the fat is placed in temporary storage when it disappears from the blood, and is only very slowly and after considerable time finally deposited in the permanent fat reservoirs or adipose tissue, it was found that the fat was very quickly deposited in the adipose tissue of the entire body, but especially and most markedly in the adipose tissue of the omentum, mesenteries, and kidneys. The pink-stained fat is abundantly and easily recovered from the chyle, the blood and the adipose tissue thus leaving no doubt as to the nature of the pink substance.

40. *Cinematomicrography of serial sections.* W. F. SCHREIBER, STACY R. GUILD, and L. G. HERRMANN, University of Michigan.

By combining photomicrographic and cinematographic methods a film has been produced on which are pictures at a low magnification of all the serial sections of an embryo, with the individual pictures so oriented with reference to each other that, when projected onto a screen by the usual moving-picture apparatus, the images overlap in much the same way that the individual wax plates used in making a model are overlapped. Whereas the successive pictures of an ordinary film gave temporal impressions, the attempt here is to give spatial impressions. It is hoped that the method will be useful as an aid in the teaching of embryology to large groups of students, especially as a supplement to the study of serial sections by the individual members of the group. The projection of the film available at the present time will constitute the major part of the presentation of this paper.

41. *The nervous system as a factor in the resistance of albino rats to parathyroidectomy.* FREDERICK S. HAMMETT (introduced by H. H. Donaldson), The Wistar Institute of Anatomy and Biology.

Studies made on the susceptibility of albino rats to acute parathyroid tetany resulting in death showed that animals which had been gentled by petting and handling were less frequently affected by removal of the parathyroids than were rats lacking this treatment. Three hundred and four rats were operated. In one group the parathyroids alone were removed, in another, the entire thyroid apparatus, and in a third the thyroid apparatus was removed in two stages, at an interval of two weeks. About 13 per cent of the gentled rats died in acute tetany after these procedures, while about 78 per cent of the not gentled rats died within forty-eight hours after operation. These results are taken to indicate that the gentling induces a condition in the nervous system such that the demand of the organism for the parathyroid secretion is lessened to the point where the rat survives though the secretion is lacking. Since the condition of high neuromuscular tension present in the rats not gentled implies a heightened metabolism of that phase of activity concerned with muscle tone, it is possible that in these rats there is thus produced a greater amount of some toxic by-product than in those gentled, and that removal of the parathyroids also removes the mechanism for the destruction or neutralization of this toxic compound and the animals consequently succumb to the excess of the hypothetical compounds so formed.

42. *An adaptation of the fire-assay method for the determination of gold and silver in animal tissues.* SAMUEL HANSON (introduced by H. M. Evans), University of California.

Methods hitherto employed in quantitative determination of gold and silver in animal tissues have not been entirely advantageous. The titration method of Voigt for the estimation of silver is inconvenient and its accuracy uncertain. The electrolytic method of Caldwell and Leavell is complicated and time-consuming. The well-known fire-assay method may be adapted to determine very small quantities of gold and silver in animal tissues with a high degree of accuracy. The tissue for estimation is dried and pulverized. Two grams of the powdered tissue is transferred to a glazed paper and thoroughly mixed with 60 grams of silver-tested litharge, 20 grams of sodium carbonate, and 15 grams of silica. The mixture is transferred to a clay crucible and covered with 10 grams of sodium carbonate. The charge is fused in the muffle at a high temperature until no suspended droplets of lead are seen. The stage is usually reached in thirty minutes. The fused material is next poured into a conical iron mold, the lead settling on the bottom of the mold, while the fused material, or slag, collects at the top. If the fusion has been properly carried out, the slag is transparent and free from particles of lead or carbon. The slag is removed and the lead hammered into the form of a cube with blunted corners. Such a lead button should weigh between 25 and 30 grams and is placed in a red-hot bone-ash cupel in the gas or electric furnace and the cupellation continued at a temperature sufficiently high to prevent heavy fumes. The cupellation may be regarded as completed when the residue suddenly loses its brightness and appears as a small bead. The bead is weighed on the assay balance to 0.01 of a milligram. After weighing the bead, the silver, if present, is dissolved and the bead reweighed. The latter weight, of course, represents the quantity of gold present, if both gold and silver are present, while the difference between the two weights corresponds to the amount of silver.

43. *On the rapidity of absorption of colloidal gold from the peritoneal cavity.*

SAMUEL HANSON (introduced by H. M. Evans), University of California.

The phenomena of absorption of dialyzable substances from the peritoneal cavity have been extensively investigated. Phenolsulphonephthalein, for example, is absorbed from the peritoneal cavity at a known rate, evidently chiefly by way of the blood capillaries, and the mechanism of its absorption can probably be satisfactorily explained by assuming that the physical processes of osmosis and filtration are operative. In the case of the absorption of colloids and suspensoids, however, the situation is different. The experimental work done with this class of substances, with the exception of the recent paper by Cunningham and Shipley, is almost exclusively qualitative. The absorption of colloidal gold furnishes an excellent opportunity to obtain quantitative data in this field, for gold may be estimated in tissues with high accuracy (see above), and its colloidal solution made according to the method of Paal is stable and largely physiologically inert. Solutions estimated to contain 1 per cent metallic gold in physiological saline were used. Injections were made into the peritoneal cavity of rats, 1 mgm. of metallic gold per 10 grams of body weight being given. After various intervals the animals were sacrificed and the

amounts of gold found in the liver determined by the fire-assay method given above. The rapidity of absorption of the gold as determined by its deposition in the liver is remarkable, 8 per cent of the amount injected being found at the end of the first hour and 22 per cent at the end of two hours. According to Dandy and Rowntree, about 50 per cent of phenolsulphonephthalein injected intraperitoneally is absorbed within one hour. This substance diffuses readily through animal membranes and its smallest particles are probably represented by the molecular dimension; the size of the gold particles is in contrast very many fold greater, yet they traverse the peritoneal boundary at a rate at least one-sixth as rapid.

44. *The development of the balancer in Amblystoma.* ROSS G. HARRISON, Yale University.

As shown by Bell in *Diemyctylus*, the development of the balancer is determined by a patch of differentiated ectoderm overlying the mandibular region. When this ectoderm is transplanted to other regions of the embryo in *Amblystoma punctatum*, even as early as the medullary-plate stage, a normally constituted, though somewhat smaller, balancer develops. If it is replaced by ectoderm from the trunk, the front of the head, or from the gill region, no balancer develops. Removal of the mandibular mesoderm does not affect the development of the balancer, if the ectoderm is healed back in place, nor does the removal of the ganglion crest (experiment of L. S. Stone), though cells from the latter lie directly under the base of the outgrowing organ and normally probably provide its mesodermal core. *Amblystoma tigrinum* lacks balancers. If, however, proper ectoderm of *A. punctatum* is transplanted to the former species, a normal balancer with mesenchyme and blood circulation develops. The reciprocal operation apparently suppresses the balancer, though only one experiment has given this result. Owing to faulty placement of the graft in others, regeneration from the host occurred. The peculiar membrane which supports the balancer develops in the strange as well as in the normal location. Its staining qualities and the imbedding of its base in mesenchyme might lead one to regard it as a dermal bone, as Latta has done. However, it is never formed except beneath the specific balancer ectoderm, and the evidence favors its interpretation as a basement membrane.

45. *Amitosis in ciliated cells.* FRANK HELVESTINE, JR. (introduced by H. E. Jordan), University of Virginia.

In 1898 v. Lenhossek and Hennequy independently expressed the opinion that the basal bodies found in ciliated cells are derivatives of the centrosome. The corollary of this hypothesis, namely, that on account of the preemption of the centrosome in the formation of basal granules, ciliated cells must necessarily proliferate by amitosis, was later expressed by Jordan ('13), and he supported this conclusion by data derived from a comparative study of the ciliated epithelium in the ductile efferentes and epididymes of a number of vertebrates.

Saguchi ('17) confirms Jordan's results as regards vertebrates, but states that in invertebrates mitosis is the exclusive mode of division of ciliated cells. He describes cells undergoing karyokinesis as either not having cilia or as losing their cilia before the process of division takes place. Saguchi concludes from his obser-

vations that basal bodies and cilia are derived from mitochondria. His descriptions and illustrations do not bear out this conclusion, but rather add to the evidence of a centrosomal origin of the basal bodies.

In my material of the gills of the fresh-water mussel, *Cyclas*, no relationship between mitochondria and basal bodies, other than spatial, is discernible. Indirect evidence supports the view of the derivation of the basal bodies from the centrosome. In this form certain ciliated cells divide extensively only by amitosis. Saguchi admits that cells of ciliated epithelium dividing by mitosis possess no cilia at the time of division and my material confirms this observation. Such cells cannot properly be called ciliated cells and it can accordingly not accurately be said that ciliated cells divide by mitosis.

In view of the agreement between Jordan ('13) and Saguchi ('17) regarding an exclusively direct method of division in ciliated cells of vertebrates, and Saguchi's failure to find in invertebrates any cells with cilia in indirect division, and my demonstration of extensive amitotic divisions in ciliated cells of *Cyclas*, the general conclusion seems warranted that ciliated cells both in vertebrates and in invertebrates divide only by amitosis.

46. *Development of the innominate artery in the pig.* CHESTER H. HEUSER, Johns Hopkins Medical School.

In a closely graded series of injected embryos ranging in length from 3.8 mm. to 40 mm. which was prepared for a study of the transformations of the aortic arches and the related vessels, the development of the innominate artery can be followed from its primitive condition until its adult form is attained. The cephalic border of the bulbous ventral aorta in the 7-mm. embryo gives rise to the large third aortic arches and the rudimentary external carotid arteries. In embryos of 8 mm. the ventral ends of the third arches carry the external carotids so that the common carotids are already indicated. In succeeding stages beyond 8 mm. the ventral portions of the third and fourth arches are united into common trunks which gradually increase in length. This trunk is especially long on the right side and is a part of the innominate artery, but as the arch of the aorta becomes established from the left fourth arch the left common carotid becomes shifted over so that it arises also from the innominate. This condition can be seen in stages of about 21 mm. In older embryos the connections remain the same, but the innominate artery increases greatly in length, as seen in stages measuring 40 mm. or more.

47. *Extirpation and transplantation of thymi in larvae of Rana pipiens.* MARGARET MORRIS HOSKINS, Medical College of Virginia.

The operations were performed by E. R. Hoskins in the spring of 1919 and the report is based on a study of preserved material. Records were kept of the growth and development of the larvae and showed no effect from the experiments in this respect. The operations were of three types: complete and unilateral extirpation of thymi and transplantation of thymic tissue from one larva to another. The grafts grow well and have the appearance of normal thymic tissue. The effect of the operations on the thymi, the spleen, and on the endocrine glands has been studied from dissections and from histological preparations. When one thymus is removed there is no compensatory hypertrophy of

the remaining one, and the engrafting of thymic tissue does not affect the thymi of the host. None of the operations affects the spleen in size of appearance. The gonads, thyroids, and parathyroids also remain unchanged. In some instances the hypophyses of thymectomized larvae appear to be hypertrophied, but this is not always the case. Histologically the hypophyses of the operated animals are normal.

48. *Embryonic myelosehisis.* (Stereo-lantern.) N. WILLIAM INGALLS, School of Medicine, Western Reserve University.

The three human embryos considered naturally fall into a series of increasing teratological and pathological severity. This also applies to the embryonic adnexa. No. 83, G. L., ca. 7 mm., condition fair, chorion quite large, villi large and numerous but somewhat altered, amnion thickened, magma excessive, cord and yolk sac small, vessels indefinite. Sacral myelosehisis extending over 2.5 mm. on summit of sacral convexity, neural folds everted, prominent and sharply defined. No. 288, G. L., ca. 12 mm., condition poor, color not very good, chorion of fair size but thin, villi not well developed, hydramnios, no exocoelom, cord small, no yolk sac found, only traces of vessels. Medullary defect measures 2.5×4 mm., extending from thoracic into sacral region. Area involved is spread out on dorsum of embryo, its surface very slightly elevated, margins irregular. In No. 46 the disturbance has been much more severe. G. L. 14.5 mm., distinctly pathological, color muddy and opaque; chorion large and haemorrhagic, villi very short and scattered, hydramnios, no exocoelom, amniotic fluid slightly turbid and viscid, neither vessels nor yolk sac to be seen, cord short and markedly distended. Extensive defect involves most of cord, secondary loss of substance; marked encephalic malformation, head very small, eyes approximated, mouth gaping, nasal and palatal deficiencies. General anasarca of embryo.

49. *The effects of various types of inanition upon growth and development, with special reference to the skeleton.* C. M. JACKSON, University of Minnesota.

According to Liebig's 'law of the minimum,' as applied to animal growth by Bunge and by Osborne and Mendel for mineral and protein factors of the diet, the deficiency of any essential factor results in failure of the growth of the body as a whole, and not in the production of abnormal tissues. However, during inanition of various types there occurs a malcorrelated growth, certain organs increasing abnormally, others decreasing, with retarded or stationary body weight.

During underfeeding of young rats on a balanced diet (deficient in calories), this disproportionate growth affects practically all organs of the body, the extent varying widely in different organs; also according to age, and length and intensity of the inanition (Jackson, Stewart, Barry). Persistent skeletal growth has likewise been observed by other investigators in underfed calves, puppies, rats, and malnourished infants.

In rats on qualitatively inadequate protein diets, Osborne and Mendel found skeletal retardation proportional to that of the whole body; but more recently Mendel and Judson ('16) found persistent skeletal growth in mice. Kudo ('21) finds markedly persistent skeletal growth in rats with restricted water supply.

Absence of essential salts results in disordered or inhibited skeletal growth, in invertebrates (sea-urchin, sponges) as well as various mammals. With calcium-poor diets, the body weight may continue increasing, while the skeleton is retarded, with 'pseudorachitic osteoporosis.' True rachitis apparently depends upon a deficiency in 'fat-soluble A' vitamine. Phosphorus deficiency likewise retards skeletal development, with histological resemblance to scorbutus (which, however, is also due to vitamine deficiency).

50. *Studies of lymph nodes. II. Response of lymph nodes to irritation.* (Lantern.) THESLE T. JOB, Loyola University School of Medicine.

By injecting subcutaneously india ink in either distilled water or weak solutions of ammonium hydroxide, a condition simulating a low grade or a virulent infection may be initiated. By this method a less complicated picture is obtained but, nevertheless, just as true as when cancer cells or bacteria are injected. Thus it can be demonstrated that, in the case of ink in water, the granules are carried by phagocytes, mainly, to the first node in the drainage line. This node becomes progressively pigmented to a solid black. Then the second node in the drainage is pigmented, and so on. This being a non-irritative process, no new lymphoid tissue is formed. If ink in ammonium hydroxide be used—the ammonia being a strong irritant—the first node in the line of drainage is only partly pigmented before the second node begins to receive pigment; the lymphatic collateral circulation about the node is enhanced and an actual building up of new lymphoid tissue is begun. The significance of these results is pointed out and a practical application made.

51. *On the origin and development of the posterior lymph hearts in anuran embryos.* (Lantern.) OTTO F. KAMPMEIER, College of Medicine, University of Illinois.

The first evidence of the beginning of the posterior lymph heart on either side is manifest in 10 to 11 mm. embryos (*Bufo vulgaris*) as an accumulation of mesenchymal cells around that part of the lateral lymphatic plexus situated just lateral to the posterior vertebral vein at the level of the eleventh spinal ganglion. By gradual distention and coalescence, the vessels of the lymphatic plexus within the area of mesenchymal accumulation become transformed into a globular chamber, the posterior lymph heart. The lymph-heart anlage becomes temporarily separated from the surrounding lymphatic network, the number and position of the points of separation being relatively constant in different individuals. Two points of junction are reestablished between lymph heart and plexus, one situated on the dorsal and the other on the ventral side of the heart; later such points of entry are increased in number. The muscular coat of the lymph heart is developed from the cells of the original mesenchymal accumulation around the lymph heart plexus.

Before the efferent valve (between lymph heart and posterior vertebral vein) is formed, blood corpuscles are found in large number in the heart cavity. There is evidence that at times the embryonic posterior lymph heart itself may function as an haemopoietic center; certain it is that during its development, it, like other embryonic lymphatics, is haemophoric, that is, transports along with its lymph flow developing blood cells to the blood stream. Not only does the number of posterior lymph hearts differ among species of Anura, but it may also differ among members of the same species, and may even be different on the two sides of the same individual.

52. *Peripheral migration and distribution of medullary cells in the absence of spinal ganglia and dorsal nerve-roots in embryos of the chick.* ALBERT KUNTZ, Saint Louis University School of Medicine.

Embryos of the chick were subjected to an operative procedure at the close of the second day of incubation (forty-eight hours) by which the neural crests and the dorsal portions of the neural tube were destroyed throughout a series of successive segments. These embryos were allowed to live until the close of the fifth day of incubation. Ventral nerve-roots are present in all segments in which the motor niduli were not destroyed. Cells of medullary origin are present in these nerve-roots and along the course of their fibers. Some of these cells advance along the visceral rami and give rise to ganglia of the sympathetic trunks, others become distributed along the nerve-fibers and give rise to neurilemma. In segments in which but a small ventral portion of the neural tube remains intact, even though ventral nerve-roots but no visceral rami are present, the primordia of the sympathetic trunks are absent.

53. *Nerve terminations in the lung of the rabbit.* (Lantern.) O. LARSELL, Northwestern University.

Sensory nerve endings are found in the epithelium of the bronchial tree and its various subdivisions as far as and including the atria. These appear on anatomical grounds to consist of three types, probably receptive to different methods of stimulation. The most constant position in which sensory terminations are present is at the point of division of the various orders of branches of the bronchial tree. Motor terminations are also present, not only in the smooth muscle fibers of the bronchi and their branches, but in the pulmonary artery and its branches, including the arterioles. A few nerve fibers are also present in the tunica media of the pulmonary veins. The sensory innervation is by fairly large myelinated fibers from the vagus. The motor innervation of the bronchial musculature appears to be of the typical preganglionic and postganglionic arrangement. The preganglionic fibers terminate in characteristic pericellular networks about the cells of the intrapulmonary ganglia, and from these nerve cell processes are given off which pass to the smooth muscle bands, to terminate in relation to the unstriated muscle cells. The source of the fibers to the pulmonary vessels has not yet been determined.

54. *The growth of the organs and systems of the single comb White Leghorn chick.*

HOMER B. LATIMER, University of Minnesota.

In plotting the gross weight of the eighty-six chicks upon age in days (from day of hatching to 251 days) the resulting curves show three phases; first a slow initial rise, then a rapid increase, and later a second period of slow growth. The curve for the females begins to fall below that of the males, beginning at about seventy or eighty days. The curves of the different organs and systems may be placed in four groups as follows:

1. Those which tend to parallel the growth of the body as a whole, or the muscles, ligamentous skeleton, digestive tract, lungs, heart, kidneys, suprarenals, and integument. The curves of the percentage weights of these organs on the net body weight show a more or less rapid decline, with the exception of the musculature which increases from about 25 per cent up to nearly 50 per cent of the net body weight.

2. This group is characterized by a rapid initial rise of the growth curve, followed by a slowing of the rate of growth. In this group are the brain and eyeballs and linear measurements (body length, etc.).

3. The ovary, oviduct, testes, and comb and wattles grow very slowly at first, followed by a rapid prepubertal rise, in both absolute and percentage weight.

4. The thymus and the feathers at first grow in weight a little more rapidly than the body, followed by a decrease in weight, both relative and absolute.

When the gross body weight is substituted for the age in days, the chief differences in the curves are a more precipitous rise at first and in some cases a loss of the second flatter part of the curve.

55. *The description of the coats of blood vessels contained in Galen's De anatomicis administrationibus, Liv. VII., Cap. V. A comment on its accuracy.* FREDERIC T. LEWIS, Harvard Medical School.

The description is as follows: Venae totius corporis ex peculiari una constant tunica; nam exterior membrana ipsis nonnunquam obhaerescens, ubi colligari quibusdam aut fulciri ac contegi desiderant, illuc solum accedit. Arteriae vero duae peculiare tunicae existunt: exterior sane qualis venae est: interior autem crassitie hujus fere quintupla, insuper durior, in transversas fibras dissoluta; exterior autem, quam etiam venae obtinent, rectis fibris, et quibusdam medioeriter obliquis, transversis nullis, contexta est. Interior arteriae tunica crassa duraque ceu cutem quandam interna superficie continent, telae araneorum manifesto persimilem, in magnis quidem arteriis perspicuam, quam nonnulli tertiam arteriae tunicae statuunt: quarta vero alia peculiaris ei nulla est, sed veluti quibusdam venarum, ita quoque arteriis alicubi obhaerescit et circumtenditur membrana tenuis contegens aut affirmans aut connectans ipsas vicinis particulis.

56. *The formation of vacuoles in the cells of tissue cultures owing to the lack of dextrose in the media.* MARGARET R. LEWIS. Carnegie Laboratory of Embryology.

Cells cultivated in media lacking dextrose show numerous vacuoles in twenty-four to forty-eight hours, after which degeneration rapidly ensues. When the usual amount of dextrose (0.25 per cent) is included in the media, the vacuolation, degeneration, and final death of the cells are retarded for several days. If a medium containing from 0.5 to 1 per cent dextrose is used, the cells continue in an apparently healthy condition for a much longer period of time, sometimes two or three weeks, during which vacuoles fail to appear. Ultimately, however, the cells in such cultures may exhibit vacuoles. Dextrose is an important part of the medium for tissue cultures, and it seems to be necessary in order to maintain the normal metabolism of the cells under the conditions of tissue cultures.

57. *The characteristics of the various types of cells found in tissue cultures from chick embryos.* WARREN H. LEWIS, Carnegie Laboratory of Embryology.

Each type of cell that migrates out of the explant onto the coverslip does so in a manner peculiar to its type. The blood cells and clasmatoocytes pursue very irregular and uncertain paths, each cell retaining its complete independence, in that they rarely adhere together to form any sort of pattern. In marked contrast to these wandering cells are the ectodermal and endodermal cells which always migrate out in the form of a sheet or membrane, the borders of the cells

adhering to their neighbors in more or less even lines. Intermediate between these two extremes are the mesenchyme, endothelial and smooth muscle cells which form loose reticuli, in that the cells tend to adhere to one another by their processes rather than by the cell borders. The cells of each type form, however, their own peculiar characteristic pattern of reticulum. Isolated ectodermal and endodermal cells occur and still more frequently isolated mesenchyme, endothelium and smooth muscle cells. Still different are the characteristic outgrowths of long multinucleated strands from the striated muscle and the long slender nerve fibers from both the sympathetic and central nervous systems. Both the muscle strands or buds and the nerve fibers have a tendency to form anastomosing plexuses, the nervous ones being more elaborate and complicated. The various other types of cells which migrate onto the coverglass do so each in a characteristic formation of characteristic cells. These characteristics both of the individual cells and of the types of growth are retained throughout the life-history of the culture, or until marked degeneration changes take place. There is no dedifferentiation after they have grown out on the coverslip, although cell division is frequent.

58. *Smooth muscle and endothelium in tissue cultures.* WARREN H. LEWIS, Carnegie Laboratory of Embryology.

Smooth muscle from the amnion and endothelium from the sinusoids of the embryonic chick liver form a somewhat similar reticulum in the cultures. They resemble one another much more than they do the ordinary mesenchyme from the subcutaneous tissue. The smooth muscle cells have a rather thick homogeneous ectoplasm and in the living cell no indications of fibrillae are to be seen unless the cells are subjected to a sudden change. The fibrillae that have been occasionally observed under such conditions were gradually lost, the ectoplasm becoming homogeneous again. On fixation under the microscope the striae and fibrillae appear as the coagulation of the ectoplasm proceeds. The fibrillae are coagulation products of a peculiar kind of ectoplasm. They are not always parallel, but may in different parts of the spread-out cells run in groups at different angles. The peculiarity of ectoplasm which causes it to coagulate into fibrillae of varying sizes is probably a molecular thing, and it is to the latter that the contractile substance owes its peculiar properties. Our observations are in entire accord with those of Mrs. Lewis on smooth muscle.

Endothelial cells often show somewhat similar striae or fibrillae on fixation. The condition is never so marked as in smooth muscle, but it suggests that there is an unusual amount of contractile substance in endothelium which is interesting in connection with recent physiological work on the contraction of the capillaries by Dale, Krogh, Bayliss, and Hooker.

59. *Preliminary remarks upon the functional variations of the normal human mammary gland.* JOSEPH McFARLAND, University of Pennsylvania.

In the study of cases of a morbid condition of the human mammary gland known as 'abnormal involution,' a variety of appearances were encountered that were very puzzling. The difficulty seemed to lie in uncertainty as to what was and was not to be regarded as normal, and part of the evolution and involution of the gland. Books and journals did little to help one out of the dilemma.

Text-books of histology, for the most part, describe and illustrate the structure of the mammary tissue in such manner as to lead one to suppose that, except at the time of lactation, all glands look alike.

With a view of finding out what variations in structure and appearance the normal mammary gland presents, about 200 apparently normal glands were collected, sectioned, and studied. From this work it has become evident that a number of structural types will have to be established, and it is believed that in the future it will be necessary to call the attention of the student to each of these types, in order that he shall not later be surprised and confused by finding that the structure of a gland that he is called upon to examine in the pathological or hospital laboratory does not correspond with what he has been taught and shown as a student.

60. *The influence of the lateral-line system in the development of the skeleton.* Roy L. MOODIE, University of Illinois, College of Medicine.

The study of the lateral-line canals in ancient Amphibia and primitive fishes shows a definite correlation with certain peripheral osseous elements of the head. This fact suggests that during development there may be a relationship between the formation of the canals and the initiation of osseous development.

Young catfishes, *Ameiurus nebulosus*, were cleared by the potash method and the relationship of both lateral-line canals and developing skull bones was studied. It was found that the lateral-line canals were all laid down prior to the deposition of any osseous material, but those bones which touch on the canals were the latest of the cranial elements to form. This indicates that the lateral-line canals have no influence on the initiation of osseous development, but that the canals do modify the form of the bones which they touch. The factor which causes the initiation of osseous deposition must be looked for elsewhere.

61. *On the specificity of regenerating limb-buds in adult newts.* C. V. MORRILL, Cornell University Medical College.

The present paper is a preliminary report on a series of transplantation experiments (still in progress) to test the specificity of regenerating limb-buds in the adult of the common spotted newt (*Diemyctylus*). The subdivisions of the problem are as follows: *a*) Will regenerating limb-buds retain their laterality if transplanted to opposite side of the body? *b*) Will a regenerating bud (e.g., from an anterior limb) retain its specificity if transplanted to the stump of a different kind of limb (e.g., to a posterior limb stump)? *c*) Is there any observable difference between autoplasmic and homoplasmic transplantations?

For the purposes of the experiment, regenerating buds were transplanted when about one-eighth of an inch long and just beginning to show indications of digits. In order to test out the various possibilities outlined above, anterior limb-buds were transplanted to the stumps of posterior limbs of the same and of opposite sides, and posterior limb-buds to posterior stumps of opposite sides. In some cases the buds used were taken from the same individual (autoplasmic), in other cases from a different one (homoplasmic). In this way seven different categories of experiments were made possible, though all have not yet been tested. The results in general show that in most cases the regenerating bud first loses most of its external and internal differentiation and becomes reduced

to a conical knob consisting of a layer of epithelium and an internal mass of more or less indifferent cells. There is undoubtedly some mingling of the tissues of transplant and stump. Subsequently a redifferentiation takes place; the bud lengthens out again and digits appear. In all cases so far examined the original laterality of the bud seems to be entirely lost, that is, the transplant develops into a limb corresponding in this regard to its new site. Regarding anterior and posterior specificity, the results are not uniform. As a rule, the original specificity is lost, but in one case an anterior limb-bud transplanted to the stump of a posterior limb of the same side (homopleural), but on a different individual (homoplastic), developed into an anterior limb. Aside from the case just cited, no differences between autoplasmic and homoplastic transplantations have as yet been detected, but the number of experiments is too small to warrant any conclusion. In all the types of experiments, reduplications occasionally appeared, as might be expected. Double limbs are of course the most common, but in two cases triple limbs developed. These are at present too young to interpret with certainty.

62. *Studies on the mammary gland. VIII. Gross changes in the mammary gland in the female albino rat during the period of involution.* FRANK J. MYERS AND J. A. MYERS, University of Minnesota.

Virgin animals of known age and weight were allowed to become pregnant, deliver, and nurse their young. In all cases the litters were weaned at the end of three weeks, after which the mammary glands of the mothers were collected at intervals ranging from six hours to five weeks. The glands were spread out on sheets of cork and cleaned according to the method previously described (Myers, '16). At the end of six hours the masses of glandular tissue are considerably enlarged. This enlargement which is probably due to the accumulation of milk continues through the forty-eight-hour stage. In the four-day stage the masses of glandular tissue have decreased considerably in size, while at the end of five days the glands are not more than one-half the size of those taken at forty-eight hours. In the stages taken at the end of two and three weeks the glands very closely simulate those of adult virgin animals. The most noticeable steps in involution occur during the latter part of the first week, and by the end of the second and third weeks the glands have returned approximately to their resting stage.

63. *Regulation of posture in the forelimb of *Amblystoma punctatum*.* J. S. NICHOLAS (introduced by R. G. Harrison), Yale University.

The limb-bud of the right side of the embryo has been subjected to rotations of 90°, dorsoanterior or clockwise and dorsoposterior or counterclockwise, in order to study the factors which cause rotation in transplanted limbs. Regulatory recovery occurs, being practically complete in the normal location and partially so in abnormal locations. As a rule, the limb moves through the shorter arc in recovering its normal posture, that is, the recovery process is generally in the reverse direction from the imparted rotation. Exceptions to this rule, occurring in dorsoposterior operations, show that occasionally growth factors increase the imparted 90° rotation, causing the limb to attempt recovery through the greater arc or in the same direction as the imparted rotation.

Irrespective of imparted rotation, girdle formation is in normal relation to the dorsoventral axis of the embryo, that is, it is never upside down, although it may be reversed in regard to the anteroposterior axis. The regulation of posture is primarily dependent upon the formation and size of the girdle. This is shown in heterotopic operations. The intrinsic musculature which grows back from the limb blastema to the girdle also apparently influences the recovery of the limb to its normal posture. The limb undergoes rotation as a whole. In contrast to this, the readjustment which occurs in the girdle is not by means of movements of the whole aggregate as shown by the position of portions of the pronephros which have been implanted with the limb-bud.

64. *The developmental topography of the thymus, with particular reference to the changes at birth and in the neonatal period.* (Lantern.) GUSTAVE J. NOBACK, University of Minnesota.

The thymus in the late fetus and stillborn child has a typical form and quite constant relations. Its lateral surfaces are convex and bulge against the medial surfaces of the lungs which rarely extend at all on its anterior surface. The thymus very rarely extends at all on the anterior surface of the right ventricle of the heart.

The thymus in liveborn infants has a typical form and relations which are similar to those found in young children. It is elongated and molded so that its anterior, lateral, and posterior surfaces bear the impress of all the organs with which it is in contact. Its lateral surfaces usually show marked convexities which are occupied by the lungs which pass over the anterior surface of this organ. Unlike the fetal thymus, it extends on the right ventricle. The change from the broad or fetal type of thymus to the elongate and molded type found in the liveborn and in the young infant bears a direct relation to the establishment of respiration. The organ is compressed from side to side by the medial surfaces of the expanding lungs. It is also compressed anteroposteriorly by the anterior borders of the lungs which advance medially and become much thickened early in the establishment of respiration.

65. *The postnatal growth and development of the female reproductive tract in the albino rat.* H. L. OSTERUD, University of Minnesota.

This study of the weights and microscopic structure of the ovaries, uterine tubes, uterus, and vagina of 125 rats (including thirty postpartum primiparae) shows that in adult virgin rats the tubes may attain a maximum growth of twenty-six times their birth weight, the ovaries seventy-four times, the vagina 138 times, and the uterus 197 times. All four organs exhibit four-phase growth curves. The most rapid growth occurs first during the first three weeks (lactation period) and later shortly after the sixtieth day of age. The prepubertal growth increase comes distinctly earlier in the uterus and vagina than in the ovaries. After maturity the variability especially in the uterine weight is astonishing (from 0.055 to 0.494 per cent of the body weight). The maximum uterine weight in virgins far exceeds that in postpartum primiparae after completed uterine involution. The uteri of these postpartum primiparae, however, display the tendency to a similar great growth if kept from the males for sufficient time. The extreme cases strongly suggest a parallelism between this great uterine growth

and ovarian activity, associated also with large hypophysis and perhaps thyroid. Failure of this great growth in some females is extremely difficult to account for except in cases of distinctly poor nutrition. Volumetric study of the ovaries offers no rôle in this uterine variability to the interstitial tissue. Definite correlation in the size of uterus and vagina is fairly evident, while the frequent apparent failure of the ovary to show similar correlation is probably due to its great cyclic fluctuation.

66. *Developmental competition in its relationship to the sex ratio.* GEORGE N. PAPANICOLAOU, Cornell University Medical College.

The average sex ratio in a stock of 3472 guinea pigs is 106.54 when the individuals born in all litters are considered. On comparing the ratios from different-size litters great discrepancies are found. In litters of one the sex ratio is 112.58; in litters of two, 112.07; in litters of three, 97.95; in litters of four, 108.73, and in litters of five, 141.02. These variations may be explained on the following principles derived from a careful analysis of the developmental conditions in guinea pigs:

1. There is a competition between developing germ-cells and embryos in the ovary and the uterus.
2. In the competition males have some advantage over the females.
3. Competition is higher in the larger litters (by a litter is meant the number of codeveloping germ-cells and embryos).
4. In litters consisting of embryos of the same sex competition is higher than in mixed litters.
5. The competition is stronger among females than among males.

In agreement with these statements there is a higher percentage of complete elimination of large litters, consisting chiefly of females than of any other large litters. This elimination produces the high sex ratio for the litters of four and five. The originally large litters in which the subsequent elimination is partial result in births of one and two. Elimination being more severe on the female members causes the production of a higher sex ratio than occurs among individuals produced in litters of three. Litters of three have the lowest sex ratio and approach nearest an expected condition, having suffered little or no prenatal mortality. This explanation is supported by a study of more than 100 litters with early partial absorptions which gave the high sex ratio of 123.37.

67. *A note on the relation of the auricle and external auditory canal to drum-membrane mechanics.* A. G. POHLMAN, Saint Louis University.

The writer presented certain comparative data at the last meeting of the Association on the problem of middle-ear mechanics. This evidence favored the 'string-telephone' theory of sound transmission and opposed the usually accepted theory of mass reactions. Practically all modern writers (Wrightson-Keith and Zimmermann excepted) agree that the drum membrane-ossicular chain route is the highly efficient one for so-called 'bone-transmitted' sound. Modern investigators of cochlear mechanics with few exceptions base the responses in the inner ear upon mass movements in the periotic fluid (functional relation of stapes basis to fenestra cochleae). It is essential that the reactions in the drum membrane to energies of optimum or minimum force be carefully studied. It appears that

the dampening-out effect of the external auditory canal upon the sound pulses entering the external meatus through diffraction is more than compensated through the action of the auricle. An explanation of the Weber phenomenon or the Rinne-negative ear test does not appear satisfactory on the basis of the mass response conception. The increased efficiency of bone-transmitted sounds (mastoid and teeth) and the decreased efficiency of air-transmitted sound in pathological conditions of the middle ear is more readily explained by the 'string-telephone' theory. This is also the case in the interpretation of instances of voluntary contraction of the M. tensor tympani and the dampening-out effect of heightened drum-membrane tension due to plus pressure in the external canal. A definite conception of drum-membrane mechanics is essential to the correct analysis of inner-ear responses.

68. *The determination of the percentage of the organic content of bone.* H. E. RADASCH, Jefferson Medical College.

The percentage of the organic substance in compact bone is given as 32 to 33 per cent. How that was determined is not apparent from the general literature. In order to determine the real percentage and to try to find out, if possible, the methods used by the early observers, experiments were made in various ways. After carefully preparing pieces of femur, tibia, and fibula, one set of pieces was weighed, then calcined, then weighed again. The loss indicated the amount of incinerable organic substance in compact bone. At twenty to sixty years the average per cent found was 40.75. In the adult cat this green weight per cent was found to be 38.32 per cent, while in the rabbit (two-thirds grown) the average was 38.90 per cent. By other methods the moisture, alcohol-soluble and ether-soluble substances were removed and the fixed organic content determined. The average amount of moisture at twenty to sixty years is 8.42 per cent and the ratio of fixed organic substance to the dried bone is only 34.92 per cent. The average amount of alcohol-soluble material is 8.46 per cent and the ratio of the fixed organic substance to the extracted bone is 32.36 per cent. The average amount of ether-soluble substance is 9.27 per cent; the ratio of the fixed organic substance to the extractable bone averages 31.34 per cent. It seems, though, that the standard weight should be that of green bone, and if this be accepted, then the organic substance averages 40.75 per cent.

69. *The distribution of the acid cells of the stomach.* H. E. RADASCH, Jefferson Medical College.

It is customary to state that the acid cells are found in the cardiac and fundal portions of the stomach, but there seems to be no definite statement as to the point or region at which they cease to exist. It was, therefore, determined to make a sort of survey of the stomach so as to see if it were possible to give any definite boundary to the acid-cell distribution and also to note any difference in distribution. For this purpose human and rabbit stomachs were fixed *in toto* and then, when dehydrating in 85 per cent alcohol, were cut. A strip $\frac{1}{2}$ inch wide of the entire lesser curvature was first cut out, then one of the entire greater curvature and one of the ventral or dorsal surface, attempting to follow the long axis of the surface. These pieces (uncut) were then completely dehydrated, cleared in cedar oil and absolute alcohol (equal parts) and then pure cedar oil,

and infiltrated in paraffin and then blocked without cutting into segments. After the paraffin had hardened the long strips were then cut into pieces $1\frac{1}{2}$ to 2 inches long (the width of the cut of a Spencer microtome) and sectioned. Such long strips may readily be cut into shorter strips by using a safety-razor blade. The stomach of the rabbit was run through whole, and if it would fit into the microtome was sectioned whole. If too large, the stomach block was cut into two pieces and sectioned in that condition. It was intended to study the stomachs in the stillborn also, but the material at hand at the time was unsatisfactory, but this will be taken up later.

70. *The sublenticular portion of the internal capsule and the thalamic radiation to the temporal lobe.* S. W. RANSON, Northwestern University Medical School.

In dissections of the internal capsule its sublenticular portion is seen to be composed of two strata. The upper stratum, immediately beneath the lentiform nucleus, is formed by the temporopontine tract. These fibers run directly lateralward into the temporal lobe. The lower stratum forms the roof of the inferior horn of the lateral ventricle and is composed for the most part of the temporothalamic fasciculus of Arnold. This bundle emerges from the thalamus near the external geniculate body, and forms a large strand directed forward in the roof of the inferior ventricular horn. A few at a time these fibers curve outward and then somewhat backward into the white matter of the temporal lobe. Another and smaller bundle of fibers can be traced from the stratum zonale in an arched course around the thalamus following the tail of the caudate nucleus. Passing through the sublenticular portion of the internal capsule, this fascicle flattens out in the roof of the inferior horn of the lateral ventricle under cover of the ependymal lining and can be traced forward to the anterior part of the temporal lobe. It lies on the ventricular surface of Arnold's bundle, and may be designated as the fasciculus thalamotemporalis arcuatus. It was seen by Probst ('05) in the brain of a monkey with an experimental lesion in the thalamus and by the same observer in the brains of microcephalic idiots.

71. *The so-called hibernating gland.* A. T. RASMUSSEN, University of Minnesota.

For this structure many other names have been proposed: adipose gland, lipid gland, cholesterin gland, brown fat, organ of hibernation, hibernating mass. From about fifty papers available, its history consists of four periods. I. 1670 to 1817, during which it was generally regarded as part of the thymus. II. 1817 to 1863, during which it was generally recognized as distinct from the thymus, but still as a haemopoietic gland. III. Since 1863 it has generally been classed as a form of adipose tissue which serves as reserved food. IV. Its internal secretory character has been emphasized during the last ten years and recently ('20) as a factor in the etiology of deficiency diseases. From the reports of others on over forty species of animals and personal examination of numerous marmots (in which this structure is prominent), it is clear that histologically there is no similarity between it and the thymus. There is no evidence of any haemopoietic function. It is also different from ordinary adipose tissue. The cells are rarely if ever unilocular. The nucleus is never flattened much. It never loses all its fat. During hibernation it supplies only about one-thirtieth of the material consumed, and hence, as far as bulk is concerned, is not an important

food reserve. While the cytoplasm of the cells is rich in small granules (in addition to the fat globules) and the organ surprisingly vascular, more careful cytological and physiological work must be done before its close relation to the suprarenal cortex, corpus luteum, or other ductless glands can be affirmed.

72. *On the growth in weight of the human body and its various parts and organs in the fetal period and its expression by empirical formulae.* RICHARD E. SCAMMON, University of Minnesota.

The growth in weight of the entire body in the fetal period presents, when plotted against total body length, a concave curve which may be expressed by the empirical formula, $Y = (aX)^b$, when Y is the weight of the body in grams, X is the total body length in cm., and a and b are empirically determined constants. The absolute weights of the trunk, the extremities, and the head also follow this course of growth and may be expressed by the same formula with modified constants. This form of growth is typical of almost all the organs of the body—certainly of the heart, kidneys, spleen, thymus, liver, stomach, pancreas, suprarenals, thyroid, eyeballs, brain, and spinal cord and, in all probability, of the lungs, testes, and uterus as well. The growth of these structures may be expressed by formulae of the same general form as that of body weight, although each appears as a minor variant of the common type. So far no evidence has been found of a grouping of these prenatal curves in categories comparable with the main classes of postnatal growth curves. Similar findings regarding the type of growth of the body and its parts and organs are obtained when weight is plotted against age in fetal months.

73. *The visual pathway and the paranasal sinuses.* J. PARSONS SCHAEFFER, Jefferson Medical College.

Recent clinical reports prompted me to undertake a more detailed study of the anatomic relationships between certain portions of the visual pathway and the paranasal sinuses than hitherto attempted in my work. An anatomic basis was sought for certain clinical manifestations. Some were cleared up, others remain obscure and require further study. It is well to recall that the optic nerve, the optic commissure, and the optic tract are formed in order by the same axones with cell bodies located in the retina and that, strictly speaking, one is dealing with a partially decussated brain tract, the fibers of which are medullated in the retro-ocular portion, but lack a neurolemma. Clinical findings are in accord with this. The portions of the visual pathway that particularly concern us here are the so-called optic nerve and the optic commissure. The great variations in size, shape, number, and type, and the variations in symmetry and asymmetry of the paranasal sinuses preclude any constancy in the topographic relationships with the optic nerve and the optic commissure. The sphenoidal sinuses and the posterior ethmoidal cells are of first importance in this connection; however, the other sinuses may be a factor. Very commonly the most intimate relationships exist.

Clinically it has been found that paranasal-sinus disease may give rise to ocular complications without external signs of orbital inflammation. Optic neuritis, neuroretinitis, phlebitis, etc., are encountered. More important, since it often occurs with but slight ophthalmoscopic change, is the occurrence of a central

scotoma. The scotoma may be unilateral or bilateral, the latter despite the fact that but one side may be affected. The above conditions may rapidly advance to a state of blindness. It is surprising, however, how rapidly these conditions clear up, even the blindness, if the optic manifestations are early recognized and paranasal-sinus treatment properly and efficiently carried out. Want of such recognition and treatment early means permanent blindness from optic-nerve atrophy. Here an appreciation of the topographic anatomy of the optic pathway and the paranasal sinuses is of the greatest importance to those dealing with the eye and the nose clinically. Apropos in this connection is the report of a prominent ophthalmologist who in consultation found a patient totally and permanently blinded by an ill-advised curettage of the sphenoidal sinus, resulting in complete destruction of the optic chiasm. The underlying anatomy of the foregoing clinical findings will be discussed. Lantern.

74. *Relation of nutrition to the oestrous cycle.* KATHARINE J. SCOTT AND HERBERT M. EVANS, University of California.

In the study of the oestrous cycles of several hundred rats, Long and Evans reported a very considerable variation in cycle length, although in over 80 per cent of some 2000 observations, cycles of six days or less were found. These observers had had occasion to note the immediate impairment of ovarian function by increased cycle length whenever experimental animals were submitted to one or more days of undernutrition. Papanicolaou and Stockard have now established similar facts on the delay of the next oestrous of guinea-pigs due to undernutrition. The suggestion was near at hand and was, in fact, made by the last-mentioned workers that the considerable variation in the length of the oestrous cycles observed in our colony of rats might be referable to chance nutritive deficiencies unintentionally and inevitably introduced by feeding table scraps. We have submitted this question to test by placing some twenty-one animals upon our usual 'table-scrap' rations and twenty-one litter mates upon a diet employed by McCollum and certified to have yielded excellent growth and reproduction in this species over a number of years. The McCollum diet consists of:

Wheat (whole)	<i>grams</i>
Casein	67.5
Whole-milk powder	15.0
Sodium chloride	10.0
Calcium carbonate	1.0
Butter fat	1.5
	5.0

The animals at all times had access to an abundance of the food and of fresh water. At the beginning of the observations all of the animals were about 100 days old and the observations to date have extended over fifty days, opportunity being thus afforded for the observation of ten or more normal oestrous cycles. During this period the rats on the standard ration made an average gain in weight of 49 grams; those on the table-scrap diet, a gain of 42 grams. The average length of all cycles observed in animals on either ration was the same and was almost exactly five days. In the case of both diets about half the animals exhibited an uninterrupted series of oestrous cycles of six days or less in length and in each group of twenty-one animals three or four individuals showed more marked

irregularity. Furthermore, two other larger groups of animals composed of ninety-five and seventy individuals, respectively, and of almost identical age but not litter mates were placed, the one on the standard ration, the other on table scraps, and a similar study of their oestrous cycles instituted. The data obtained were concordant with the above. It cannot be considered, therefore, that the irregularity which may be observed in the lengths of the oestrous cycles of young adult rats is always due to nutritive deficiency. We would not by this statement mean to deny the great importance of nutrition in maintaining the oestrous rhythm. Studies of the effect on the oestrous rhythm of experimental undernutrition both qualitative and quantitative are under way.

75. *The development of the pharynx, and the histology of its adult derivatives, in turtles.* RALPH F. SHANER, Harvard Medical School.

The pharynx of *Chrysemys marginata* develops five pouches. The last pouch, with the postbranchial body, arises from a common stem. There are six aortic arches and five nerve placodes. The second, third, and fourth pouches end secondarily in a common cervical sinus. The first three pouches have patent clefts. From the first pouch develops the auditory tube and the tympanic-mastoid cavity. The second bears a dorsal knob of doubtful significance, which vanishes with it. The third and fourth develop persisting dorsal and ventral outgrowths. The fifth develops a transient dorsal (thymic) rudiment and then disappears; the postbranchial is then attached to the fourth pouch. The thyroid gland develops entirely from a median ventral diverticulum. The dorsal and ventral outgrowths of the third pouch separate off as a single independent complex closely adherent to the carotid artery. The dorsal moiety becomes a large, lobulated, persistent, anterior thymus; the ventral one is transformed into an anterior parathyroid, which is enclosed within the adult anterior thymus. The two outgrowths of the fourth pouch and the postbranchial body separate off as another independent complex, closely adherent to the systemic arch. The dorsal outgrowth persists as a variable posterior thymus; the ventral as a large posterior parathyroid. The postbranchial body develops chiefly on the left side; it breaks up into numerous secretory vesicles. The three organs constitute the tiny aortic body, which appears in the adult, attached to the aorta. The lobules of each thymus are divided into cortex and medulla, the latter containing thymic corpuscles. Each parathyroid is made up of cords of epithelial cells, surrounded by vascular sinusoids. The postbranchial vesicles are of two types and contain a definite secretion.

76. *The presence of a head cavity in a human embryo of 4 mm.* JOSEPH L. SHELL-SHEAR (introduced by G. L. Streeter).

The cavity is situated immediately posterior to the otic vesicle and mesial to the glossopharyngeal complex, and probably corresponds to Van Wijhe's 1st post-otic segment. Spindle-shaped cells arising from it are continuous with a clump of cells of a similar character situated mesial to the vagus complex. From this latter group of cells a migration is taking place which passes posterior to the vagus apparatus and is interpreted as the migration of the hypoglossal musculature.

The cavity is regarded as homologous with the head cavities which give rise to the eye musculature. This type of cavity is peculiar to the median somatic or axial mesoderm and distinct from the coelom which is formed by a splitting of the lateral somatic mesoderm.

77. *On the reaction of the living blood cells to dyes.* M. E. SIMPSON (introduced by H. M. Evans), University of California.

A drop of blood was caught on a cover and immediately brought in contact with a slide on which was a thin, dry film of dye. The method has been previously employed by Pappenheim, Rosin, and Bibergeil. Somewhat less than two hundred dye substances were carefully studied. The following generalizations may be made:

1. The dyes frequently collect in a definite set of granules, 'the segregation apparatus,' which can be differentiated from, 1) refractile granules (probably lipid); 2) degeneration vacuoles; 3) specific granules, and, 4) mitochondria.

2. With some dyes a certain proportion of the granules enlarge rapidly, the vacuolar structures resulting therefrom having been described by Ferrata and termed 'plasmosomes.' But Arnold has used this term much more widely. Rosin and Bibergeil called them 'dye sphere formations.' They evidently correspond to what Renault termed 'grains de ségrégation' in the connective-tissue cells. Dubrueil called them 'vacuoles à grains de ségrégation;' Hammar referred to them as 'purpurgranula,' whereas Evans and Scott, in their study of the reaction of connective tissues to vital stains, described the same system of structures as 'the vacuolar apparatus.'

The segregation apparatus may be considered as a reaction on the part of the living cell for the purpose of segregating and isolating various foreign materials forced upon it. The ability to thus segregate dyes is common to all the white cells of the blood, but the extent of the segregation apparatus is characteristic for each cell type and may be used as a valuable point of distinction between the different kinds of mononuclear cells. The transitionals of Ehrlich or monocytes of Naegeli show the reaction to the greatest degree. Probably all dye groups contain members which would be handled in this way by the cell. The reaction is perhaps given most typically by certain of the oxazine, thiazine and azine dyes, but dyes showing the widest variation in chemical and physical properties appear to give this response.

78. *The ingestion of melanin pigment granules by tissue culture cells grown from the embryo chick in Locke-Lewis solution.* DAVID T. SMITH (introduced by W. H. Lewis), Carnegie Laboratory of Embryology.

In cultures of chick embryos, melanin pigment granules from the retina of the chick, pig, dog, and man (newborn child) were taken in by clasmatocytes, fibroblasts, endothelial cells, white blood cells, and cells from lung, liver, kidney, intestine, and amnion by a process which appears quite different from that by which the amoeba ingest food. Peripheral nerve cells, striated muscle cells, and red blood cells did not ingest the granules. When a granule was free in the culture fluid it exhibited both Brownian movement and an actual progression from place to place; when attached to the cell wall it was motionless; after passing into the cytoplasm it displayed the jerky motion characteristic of pigment

granules in the true pigment cells, and finally, when a vacuole developed about a granule it reverted to Brownian motion. The granules were not taken into preformed vacuoles; but later, as they moved back and forth in the cytoplasm, a vacuole developed about each one or about each small clump of granules. The granules then exhibited Brownian movement, became swollen, disintegrated, and were reduced to debris.

Granules in the true pigment-producing cells are always individual and discrete bodies of about the same size and shape. The individuality and discreteness are common properties, but size and shape vary in cells of different origins. The granules in normal pigmented cells are never found clumped into vacuoles or broken up into debris. It can therefore be determined by the appearance of the granules whether they have been produced or ingested by the cell. This fact should help us to settle the old question of pigment-producing versus pigment-carrying cells.

79. *Some modifications induced by parabiotic union of the hypophysectomized to the normal tadpole.* PHILIP E. SMITH, University of California.

It appears of interest to determine whether the disturbances resulting from early hypophysectomy in the tadpole may be modified by a vascular interchange between the normal and the pituitaryless individuals, and to observe any compensatory alterations that may occur in the normal member of the pair. Hypophysectomized individuals were united at an early stage (5 mm.) to normal larvae. Both members of four pairs completed metamorphosis, and several pairs reached a nearly maximal larval size. In every case the pigmentary and endocrine disturbances typical of hypophysectomy were modified. Albinism, though evident, was only partial. Examination of the living animal and of cutaneous whole mounts revealed the fact that the xantholeucophores were not as broadly expanded, the epidermal melanophores not as scanty in number, as poor in melanin content, nor as contracted as in the typical hypophysis-free tadpole. The thyroids of the albinous member, instead of being diminutive, as would otherwise have been the case, were nearly normal in size, while those of the normal mate exhibited a slight hypertrophy. The adrenal cortex while reduced did not appear to suffer the same great reduction as that which normally occurs in the typical Albino. The vascular interchange did not modify the greatly reduced and atypical neural lobe of the Albino, nor did it appear to have caused an hypertrophy of the hypophysis of the normal member of the pair.

80. *Upon the essentiality of the buccal component of the hypophysis for the continuance of life.* PHILIP E. SMITH, University of California.

The above-mentioned parabiotic pairs were united in several ways, one of which, a union of the corresponding sides of the tail-stalks, is of especial interest here. Two such pairs completed metamorphosis. It is obvious that the attachments would be severed by metamorphosis. One pair completely separated; in the other an atrophic connecting strand persisted. Both members of each pair displayed the usual activity up to three or four days prior to the completion of metamorphosis. The hypophysectomized members then became more sluggish and exhibited a slowed respiration. One of them died just after the separation, the other just before the separation would have taken place. The separation

in no way embarrassed the normal member of either pair. These members displayed their usual activity. In two other pairs joined by their heads, metamorphosis did not result in the death of the hypophysectomized specimen, probable due to the persistence of the vascular interchange.

Mammalian experimentation appears to have established the fact that the neural lobe is not essential to life. The essentiality of the anterior lobe has been questioned, death from its removal being referred by some to injury of the neighboring structures, not to hypophysial deficiency. In this experiment there was no injury to the brain or other neighboring structures, yet the animals promptly died when, in the adult stage, they were deprived of the secretion of the buccal hypophysis. The functional similarity which experimental work has shown to exist between the parts of the amphibian and mammalian hypophyses makes it highly probable that this component is essential for life in the mammal as well.

81. *Does the administration of anterior lobe to the tadpole produce an effect similar to that obtained from thyroid feeding?* PHILIP E. SMITH AND GARNETT CHENEY, University of California.

In a recent paper Hoskins and Hoskins have advanced evidence showing that the administration of a commercial anterior-lobe preparation to the normal and thyroidless tadpole gives an effect similar to that which is characteristic for thyroid administration, i. e., causes metamorphosis. This evidence would indicate that the anterior lobe and the thyroid are in this respect functionally similar, and so supports the hypothesis that they may function vicariously. These results are at variance with those obtained by fresh anterior-lobe feeding. We have found that the feeding of the particular commercial preparation used by the Hoskinses gives the results obtained by them. Two other commercial preparations, the dried gland prepared in this laboratory and the fresh gland, failed to give similar effects. Analysis by Doctor Kendall showed that this commercial preparation contained iodine greatly in excess of the normal amount.

Iodine as KI was added to the dried gland prepared in this laboratory in a sufficient amount to give an iodine content identical with this commercial product. Tadpoles receiving this substance did not exhibit a decisive acceleration of metamorphosis. In another case iodine as thyroxin iodine was added in identical amounts. Normal and thyroidless tadpoles receiving this substance paralleled in development the animals fed with the commercial preparation in question. The evidence indicates that a similarity of response is not evoked by thyroid and hypophysial administration. The anterior-lobe preparation used by the Hoskinses contained an unusual amount of iodine and displayed an altogether unique activity.

82. *On the presence of longitudinal collector nerves in the tail of the skate and dogfish.* CARL CASKEY SPEIDEL, University of Virginia.

In the tail of the skate there are present four longitudinal collecting nerve trunks. These extend throughout the tail, but are not present in the body proper. They are located two on each side of the vertebral column, close to the bodies of the vertebrae. The dorsal collector on each side is opposite the junction of the neural arch and centrum of each vertebra. The ventral collector on each side is opposite the junction of the haemal arch and centrum of each vertebra.

These collectors are fed regularly by the spinal nerves according to the following system: the ventral root from the spinal cord unites with the dorsal root emerging from the succeeding intervertebral foramen. From this junction emerge two rami, one of which connects with the dorsal collector, the other with the ventral collector. Branches to the muscles and electric organs are given off from these rami. No branches were found from the collectors to the electric organs. Small branches from the collecting trunks to the blood-vessels led to the supposition that they might represent a sort of primitive sympathetic system, a forerunner of the sympathetic system of higher vertebrates. Osmic-acid preparations, however, showed no non-medullated fibers. All the nerve fibers were medullated. Similar collecting nerve trunks have been found in the tail of the dogfish and shark, although the connections with the spinal nerves were somewhat different.

83. *Comparative study of large irregular cells in the spinal cord of other fishes homologous to the giant glandular cells in the spinal cord of the skates.* CARL CASKEY SPEIDEL, University of Virginia.

In the caudal portion of the spinal cord of the skate there are present large irregular cells of glandular character. Cells homologous to these have been found in more than thirty genera of fishes. These include both fresh- and salt-water forms, and represent the elasmobranch, teleost, and ganoid fishes. In three genera of fishes the cells were not found. In most of the forms the cells are neither so conspicuous nor so active as in the skate. Granular secretion is usually scanty or lacking. A form of special interest is the summer flounder in which the cells are extraordinarily large and numerous. This unusual type of cell, then, may be said to occur in the great majority of fishes, reaching its greatest degree of development in the skate and flounder. A doubtful homologous cell has been found in the ventral nerve cord of the lobster, but not in the horse-shoe crab. In none of the vertebrates higher than fishes have the cells been found.

84. *Experiments on the development of the cranial ganglion and the lateral-line sense organs in Amblystoma.* L. S. STONE (introduced by R. G. Harrison), Yale University.

These experiments involve the removal of placodes and neural-crest cells. In normal development the crest cells migrate ventrally over the mesoderm of the visceral arches, around which they wrap themselves, and finally become situated on their median surfaces, where they form the visceral skeleton. The appearance of a few mesodermal yolk granules among the crest-cell aggregations gives one the impression that there may be a slight mesodermal contribution to the visceral skeleton. When the crest cells are removed, a few cases show an incomplete formation of the visceral skeleton, but no defects in the ganglionic components are observed due either to the fact that they may take no part in the formation of the ganglia or to the difficulty in eliminating the crest cells on account of their persistent ability to regenerate. All groups of lateral-line organs have separate primordia, except possibly the maxillary group, which may be a branch of the ventral hyomandibular. When sheets of ectoderm, taken anterior and posterior to the position of the ear, are removed at the closure of the neural folds, the body lines, occipital and supra-orbital primordia and their corresponding

lateral line ganglia are absent. Removal of the epibranchial placodes of VII, IX, and X produces small ganglia apparently lacking visceral sensory and cutaneous components. Placodes of vagus and facial lateral-line ganglia interchanged produce in their new positions irregular groups of many sense organs innervated by fibers from lateral-line ganglia in the transplanted region.

85. *A well-preserved human embryo of the presomite period.* GEORGE L. STREETER, Department of Embryology, Carnegie Institute of Washington.

Lantern slides will be shown of a young human embryo which was found at autopsy by Dr. H. G. Weiskotten, of Syracuse University. The patient, twenty years old, having skipped one menstrual period, died after an extensive pelvic retroperitoneal hemorrhage, presumably originating from an attempt to induce an abortion. The embedded ovum was found in the fundus of the uterus, and, together with the adjacent portion of the uterine wall, was placed in 10 per cent formalin seven and one-quarter hours after the death of the patient. Due to the handling of the specimen during its removal, the ovum and its decidual capsule were partially loosened from the implantation site and apparently flattened, but otherwise the specimen appears to be normal and in an excellent state of preservation. The external diameters of the decidual capsule are $16 \times 12 \times 5.3$ mm. The diameters of the chorionic cavity are $9 \times 7.3 \times 2$ mm. The embryo, i.e., the yolk-sac and amniotic vesicle combined, measures $2.2 \times 2.1 \times 1.2$ mm. The greatest width of the embryonic shield is slightly less than 1 mm. On account of its being bent upon itself, the length cannot as yet be accurately stated. The general form of the embryo and the character of the chorionic villi will be shown in the slides.

86. *The order, time, and rate of ossification of the skeleton. II. Mammals.* R. M. STRONG, Loyola University School of Medicine.

The white rat has been the type used for most of the mammalian portion of this work to date. The results contain too many details for the space allowed in an abstract. Some of them are mentioned here: A series of features of the skull, girdles, and long bones have been studied. Stages from the first appearance of ossification to senility (730 days) have been compared.

As in the bird, beginning ossification occurs in several bones at about the same time. The first ossification stages occur fully a week later in rat embryos than in the chick. At seventeen days and fifty-five minutes after insemination, ossification was found well started in the mandible, clavicles, and in the second to eleventh ribs. It had also begun in the maxilla, palatine, premaxilla, orbital portion of the frontal, humerus, radius, and ulna. The scapula showed ossification at seventeen days and eight and one-fourth hours. This had extended to a large portion of the spine several hours later. An ossification center was found in the coracoid process at three days after birth, and fusion with the scapula early in the fourth month. Ossification centers appear in the ilium, femur, tibia, and fibula at eighteen days nine and a half hours. They appear in the ischium and pubis at nineteen days eight and three-fourths hours. In the same embryo, the deltoid crest is well started, and it resembles the adult form a day later. Except for changes in size and general form, the rat skeleton is essentially mature at the end of the first year. Only slight stages take place after the third month.

87. *Situs inversus in double trout.* F. H. SWETT, Yale University.

Examination has been made of the situs viscerum in fifteen double trout embryos and the findings resolve themselves into the following classes: In nine cases the situs viscerum of both components is normal; in one, that of A (the right twin) is reversed; in two, B (the left twin) is reversed, and in three B is normal, A of indeterminate situs. One of the cases which shows situs inversus in component B is of the auto site-parasite type and it is the parasite which is reversed. A definite correlation between the amount of external or internal doubling and the occurrence of situs inversus cannot be demonstrated.

88. *The relation of the pars intermedia of the hypophysis and the pineal gland to pigmentation changes in anuran larvae.* W. W. SWINGLE (introduced by R. G. Harrison), Yale University.

Homoplastic and heteroplastic transplants of the pars intermedia of the hypophysis from adult frogs of the species *Rana catesbeiana*, *Rana clamitans*, and *Rana pipiens* were made into bullfrog tadpoles of various ages and sizes. The effect upon growth and metamorphosis of the animals was negative, but pigmentation changes following transplantation of the tissue were very marked. Within twenty-four hours after engrafting the pars intermedia either intraperitoneally or into the abdominal lymph spaces the larvae became deeply pigmented, changing color from a light yellow to almost black. The color change is due to marked expansion of the melanophores of the skin, though the deeper-lying pigment cells of the tadpole also expand. The increased pigmentation lasts as long as the engrafted pituitary tissue remains functional and is not resorbed. Following resorption of the graft, the animals resume normal coloration. The environment apparently plays no part in the color change following transplantation of the pars intermedia; the change is due to the stimulating effect of the hormone either directly upon the melanophores or else indirectly through the intermediation of the nervous system.

There is a possible interrelationship of the pars intermedia to the pineal gland in the production of pigmentation changes in anuran larvae. Darkly pigmented tadpoles engrafted with the pineal gland of reptiles (*Chelonia*) change color within an hour following transplantation; the expanded melanophores contract and the animals become lightly pigmented. This condition persists for several hours; then slowly normal pigmentation is resumed. Similar changes follow introduction of desiccated mammalian pineal tissue into body cavity.

89. *Mammalian pubic metamorphosis.* (Stereo-lantern.) T. WINGATE TODD, Western Reserve University.

In comparing skeletal growth and metamorphosis of man with similar features in other mammals, it is necessary to utilize some standard subdivision of the total life period. In our present work the best subdivision is the following:

- 1st life period. Terminates in complete union of the acetabular elements.
- 2nd life period. Terminates in complete union of epiphyses with long bones.
- 3rd life period. Terminates in complete union of epiphyses with vertebral centra.
- 4th life period. Between the termination of the 3rd and the commencement of the 5th period.

5th life period. Commences with lipping of the glenoid margins of the scapulae.

6th life period. Commencement of senile (quasipathological) erosions and osteophytic growths at joints, and senile textures of bones.

These features, unlike eruption of teeth, closure of cranial sutures and others not here mentioned, present definite relationships to the total life period. Compared with each other they do not represent even approximately equal time relationships. Judged by these standards, it is possible to observe the delay in commencement and still more in completion of pubic metamorphosis as evidenced by higher mammals and by man. At the same time the gradual evolution of the features of this metamorphosis can be studied. Its progressive features are exemplified in members of some orders, other members of which show retrograde conditions. Man falls into the latter group.

90. *The skull as a closed box.* LEWIS H. WEED AND WALTER HUGHSON, Johns Hopkins Medical School.

The experiments of Weed and McKibben, reported two years ago, demonstrated that the pressure of the cerebrospinal fluid may be markedly lowered and frequently reduced to negative values by appropriate intravenous injections of strongly hypertonic solutions. These findings suggested that the cerebrospinal axis was enclosed within a rigid system, but absolute proof of the 'closed-box' character of the coverings was lacking. Experiments recently performed indicate that if the bony calvarium on one side be removed without opening the dura mater and if the pressure of the cerebrospinal fluid be taken, repeated intravenous injections of strongly hypertonic solutions fail to reduce the pressure to below zero. Likewise, if the bony calvarium on one side be opened and then temporarily sealed, appropriate intravenous injections of the hypertonic solutions will reduce the pressure of the cerebrospinal fluid to negative readings. Under these circumstances, opening the cranial cavity by removal of the sealing device will cause the pressure of the fluid to become immediately positive, the level of the positive pressure being determined by the hydrostatic height of the brain above the needle. These experiments can be explained only upon the hypothesis that within minimal limits the cranium and vertebral canal form a closed system within which lies the central nervous system.

DEMONSTRATIONS

1. *The motor cortex of the brain of the sheep.* CHARLES BAGLEY, JR., Johns Hopkins University.
2. *The development of connective tissue.* GEORGE A. BAITSELL, Yale University.
3. *a—De-electrification of paraffin ribbon. b—Differential bone stains for macroscopic transparent preparations.* O. V. BATSON, University of Wisconsin.
4. *Injection of blood vessels of the lung of the chick during third day of incubation to show the origin of pulmonary veins.* CHARLES E. BUELL, JR. (introduced by Floreneſ R. Sabin), Johns Hopkins Medical School.
5. *Plates of a radiographic atlas of anatomy.* H. S. BURR, Yale University, School of Medicine.

6. *Model of the principal fiber tracts of the central nervous system.* J. L. JACKOWITZ (introduced by H. S. Burr), Yale University, School of Medicine.
7. *Microscopic slides, drawings and graphs illustrating bone, muscle and joint origin in the thigh of the pig (Sus scrofa).* EBEN J. CAREY, Marquette School of Medicine.
8. *Sections illustrating the effect of stress and strain upon the healing of bone injuries.* ELIOT R. CLARK and RALPH R. WILSON, University of Missouri.
9. *Digestion of different proteins by the mesenchyme and its derivatives in the tadpole.* VERA DANCHAKOFF, Columbia University.
10. *Various techniques used in scientific illustration.* ERWIN F. FABER, University of Pennsylvania.
11. *Preparations showing the absorption and assimilation of fat.* SIMON H. GAGE, Cornell University.
12. *A case of hermaphroditism in the pig.* HARLEY N. GOULD, Lake Forest College.
13. *a—Thyreo-parathyroidectomized and parathyroidectomized albino rats. b—Effects of removal of the thyroid apparatus on bone growth of albino rate. c—Malformation of the femur and humerus accompanying abdominal tumor in a female albino rat.* FREDERICK S. HAMMETT, The Wistar Institute.
14. *Injected pig embryos cleared by the Spalteholz method to show the development of the innominate artery.* CHESTER H. HEUSER, Johns Hopkins Medical School.
15. *Demonstration of the value of x-ray in anatomical teaching and research.* EBEN C. HILL, Johns Hopkins Medical School.
16. *Regenerative processes in the spinal cord of frog larvae severed previous to metamorphosis.* DAVENPORT HOOKER, University of Pittsburgh.
17. *Section cutting of the dental tissues by means of the ether-vaporizing microtome.* A. HOPEWELL-SMITH, University of Pennsylvania.
18. *Stereoscopic photographs of human embryos.* N. WILLIAMS INGALLS, School of Medicine, Western Reserve University.
19. *Sections of chick embryos showing ganglia of the sympathetic trunks derived from cells which advanced peripherally along the fibers of the ventral nerve-roots in segments in which the spinal ganglia and dorsal nerve-roots are absent.* ALBERT KUNTZ, St. Louis University.
20. *A method for preserving cadavers in the dissecting-room.* FREDERIC P. LORD, Dartmouth Medical School.
21. *Histological preparations showing various stages in lactation and subsequent involution of the mammary gland in the albino rat.* L. M. A. MAEDER (introduced by C. M. Jackson), University of Minnesota.
22. *Microscopic sections showing functional variations in normal human mammary glands.* JOSEPH MCFARLAND, University of Pennsylvania.
23. *Cleared preparations illustrating the involution of the mammary gland in the female albino rat.* FRANK J. MYERS (introduced by J. A. Myers), University of Minnesota.
24. *a—A graph illustrating simple formulae for correlating crown-heel and crown-rump length in fetal life. b—Material illustrating the developmental topography of the thymus with particular reference to the changes at birth and in the neonatal period.* GUSTAVE J. NOBACK, University of Minnesota.
25. *a—Wax reconstruction of the nuclear masses in the brain stem of a sheep.* JAMES W. PAPEZ, Cornell University.

26. *b*—*Wax reconstruction of the olivary nuclei of the sheep, rabbit, dog and bear.* JAMES W. PAPEZ, Cornell University.

The inferior olivary nucleus of the rabbit, sheep, dog and bear is divisible into the dorsal, ventral and intermediate olivary nuclei and the olivary sac. The dorsal nucleus is an oval plate that lies dorsal to the sac and is secondarily separated from its dorsal lip. The ventral nucleus is a thick oval plate that extends the entire length and with the intermediate nucleus forms the caudal end of the olivary complex. Orally its medial margin is secondarily separated from the ventral lip of the sac. The intermediate nucleus is formed of three parts; the paramedian plate at the caudal end of the complex united with the ventral nucleus, the intermediate plate extending laterally to the caudal end of the ventral lip of the sac, and the olivary bridge extending from the paramedian plate laterally to join the narrow caudal ends of the dorsal nucleus and sac. The olivary sac is a simple oval sac, compressed dorsoventrally with its opening towards the mid line. The sac is situated in the oral portion of the olivary complex where it intervenes between the dorsal and ventral nuclei. The hypoglossus nerve perforates the dorsal nucleus and lateral end of bridge and in the rabbit also the sac. The larger oral portion of the olivary nucleus appears to have been rotated laterally in the expanded portion of the bulb while the caudal portion has retained a more fixed position oral to the decussation of the cerebrospinal tracts.

27. *Materials in a case of multiple atresia of the jejunum of a young child suggesting etiological factors.* C. W. M. POYNTER, University of Nebraska Medical College.

28. *a*—*Field graphs, curves and charts illustrating the growth of the various external dimensions of the human body in the fetal period.* L. A. CALKINS (introduced by R. E. Scammon), University of Minnesota.

29. *b*—*Material illustrating the growth of the brain and its parts and of the spinal cord in the fetal period of man.* H. L. DUNN (introduced by R. E. Scammon), University of Minnesota.

30. *c*—*Graphs and charts illustrating the growth in weight of the body as a whole and its various parts and organs in the fetal period of man.* R. E. SCAMMON, University of Minnesota.

31. *Histological preparations of experimentally doubly-ligated blood vessels, showing the fate of the contained blood and the behavior of the intima.* J. PARSONS SCHAEFFER and H. E. RADASCH, Jefferson Medical College.

32. *A series of graphs illustrating the changes in the form of the thorax at birth and in the neonatal period.* RICHARD E. SCAMMON and WILLIAM E. RUCKER, University of Minnesota.

These graphs are based upon measurements of the chest in fetuses, full-term children and a series of infants less than two weeks old. The horizontal chest circumference is greatly increased with the first inspiration, but in the course of the first 24 hours enters a period of decrease which continues for three or four days. Following this is a second phase of circumference gain which continues throughout the remainder of the period of observation. The diameters of the thorax undergo changes similar to those of the chest circumference. The thoracic index stands below 90 in the latter part of the fetal period, but it rises to an average of about 106 with the establishment of respiration, and then drops

to about 102 in the first 24 hours. Thereafter it declines irregularly to about 100.5 in the middle of the second postnatal week.

33. *Cinematographic of serial sections.* W. F. SCHREIBER, STACY R. GUILD, and L. G. HERRMANN, Anatomical Laboratory, University of Michigan.

34. *Histological preparations showing the effects of inanition upon the development and structure of the testis in the albino rat.* DAVID M. SIPERSTEIN (introduced by C. M. Jackson), University of Minnesota.

35. *Model illustrating the effect on the growth of the sacrum following early removal of the posterior limb-bud in chick embryos.* R. G. SPURLING (introduced by E. R. Clark), University of Missouri.

36. *Charts showing the weight of the ovaries during the reproductive cycle in albino rats.* a—During gestation; b—During normal lactation; c—In females deprived of their litter at birth. J. M. STOTSBERG, The Wistar Institute.

37. *Unique case of ectopic pregnancy.* G. L. STREETER, Carnegie Laboratory of Embryology.

A chorionic sac containing a normal human embryo of about eight weeks development which was obtained by operation from the subcutaneous tissue superficial to the rectus muscle midway between the umbilicus and the pubis.

38. *Cleared embryos and postembryonic stages.* R. M. STRONG, Loyola University School of Medicine.

39. *Free costal bars of the epistropheus of an adult man.* PAUL K. WEBB (introduced by R. J. Terry), Washington University School of Medicine.

This rare variation is interpreted as further evidence of the tendency to reduction and special modification of the vertebrae at the cranial end of the column.

40. *Anomalous right subclavian artery in man.* WILLIAM A. HUDSON (introduced by R. J. Terry), Washington University School of Medicine.

The relation of this variant to the oesophagus and the presence of an 'aneurysmal' swelling at its origin have been regarded as possibly causing dysphagia in the subject; it is suggested that the pressure of the anomalous vessel upon the thoracic duct may be a factor in the incidence of slow starvation recorded in connection with this variation. Absence of a right recurrent nerve and origin of the inferior laryngeal directly from the vagus is of practical interest in the operations for goitre.

41. *Chondrocranium of *Caluromys philander*.* Wax plate model from a 17 mm. embryo. WALCOTT DENISON and R. J. TERRY, Washington University School of Medicine.

Of special interest are: the shallow pituitary fossa and rudimentary dorsum sellae; absence of a true optic foramen; high degree of independence of the nasal capsules; presence of paired vomers; an unpaired nasal ossicle (os carunculae).

42. *Vitally stained polymorphonuclear leucocytes in the placenta.* GEORGE B. WISLOCKI, Johns Hopkins Medical School.

CONSTITUTION

ARTICLE I

Section 1. The name of the Society shall be "The American Association of Anatomists."

Sec. 2. The purpose of the Association shall be the advancement of anatomical science.

ARTICLE II

Section 1. The officers of the Association shall consist of a President, a Vice-President, and a Secretary, who shall also act as Treasurer. The President and the Vice-President shall be elected for two years, the Secretary for four years. In case of absence of the President and Vice-President, the senior member of the Executive Committee shall preside. The election of all the officers shall be by ballot at the annual meeting of the Association and their official term shall commence with the close of the annual meeting.

Sec. 2. At the annual meeting next preceding an election, the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than two months before the annual meeting at which the election is to take place. It shall be the duty of the Secretary to mail the list to all members of the Association at least one month before the annual meeting. Additional names for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE III

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of eleven members, including the officers. Two members of the Executive Committee shall be elected annually and, so far as possible, election of members of the Executive Committee shall be in proportion to the geographical distribution of members. Five shall constitute a quorum of the Executive Committee.

ARTICLE IV

The Association shall meet at least annually, the time and place to be determined by the Executive Committee. The annual meeting for the election of officers shall be the meeting of convocation week, or in case this is not held, the first meeting after the new year.

ARTICLE V

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences, and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recom-

mendation by a list of the candidate's publications, together with references. Their election by the Executive Committee, to be effective, shall be ratified by the Association in open meeting.

Sec. 2. Honorary members may be elected from those who have distinguished themselves in anatomical research. Nominations by the Executive Committee must be unanimous and their proposal with a reason for recommendations shall be presented to the Association at an annual meeting, a three-fourths vote of members present being necessary for an election.

ARTICLE VI

The annual dues shall be seven dollars. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeting of the Association, but may be reinstated at the discretion of the Executive Committee on payment of arrears.

ARTICLE VII

Section 1. Twenty members shall constitute a quorum for the transaction of business.

Sec. 2. Any change in the constitution of the Association must be presented in writing at one annual meeting in order to receive consideration and be acted upon at the next annual meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

Sec. 3. The ruling of the Chairman shall be in accordance with "Robert's Rules of Order."

The orders adopted by this Association, which read as follows, have not been altered:

Newly elected members must qualify by payment of dues for one year within thirty days after election.

The maximum limit of time for the reading of papers shall be fifteen minutes.

The Secretary and Treasurer shall be allowed his traveling expenses and the sum of \$10 toward the payment of his hotel bill, at each session of the Association.

That the Association discontinue the separate publication of its proceedings and that the Anatomical Record be sent to each member of the Association, on payment of the Annual Dues, this journal to publish the proceedings of the Association.

AMERICAN ASSOCIATION OF ANATOMISTS

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BOOKS RECEIVED

THE BLIND: THEIR CONDITION AND THE WORK BEING DONE FOR THEM IN THE UNITED STATES, by Harry Best, Ph. D., 764 pages, The Macmillan Company, New York, 1919.

Foreword. In the present study the field of inquiry in respect to the blind has been limited to the United States, except in so far as an account is necessary of the operations in foreign countries in the way of affording instruction to blind children and of devising a system of raised print, as an introduction to the work in this country. References are accordingly only to American sources, save as to a restricted number of publications in England dealing with the two subjects mentioned, with popular conceptions regarding the blind, and occasionally with other matters.

EMBRYOLOGY OF THE CHICK, by Bradley M. Patten, Western Reserve University, 168 pages, 182 figures, P. Blakiston's Son & Company, Philadelphia, 1920.

Preface. The fact that most courses in vertebrate embryology deal to a greater or lesser extent with the chick seems to warrant the treatment of its development in a book designed primarily for the beginning student. To a student beginning the study of embryology the very abundance of information available in the literature of the subject is confusing and discouraging. He is unable to cull the essentials and fit them together in their proper relationships and is likely to become hopelessly lost in a maze of details. This book was written in an effort to set forth for him in brief and simple form the early embryology of the chick. It does not purport to treat the subject from the comparative view point, nor to be a reference work. If it helps the student to grasp the structure of the embryos, and the sequence and significance of the processes he encounters in his work on the chick, and thereby conserves the time of the instructor for interpretation of the broader principles of embryology it will have served the purpose for which it was written.

THE STORY OF THE AMERICAN RED CROSS IN ITALY, by Charles M. Blakewell, 254 pages, 20 Illustrations, The Macmillan Company, New York, 1920.

Introduction. The purpose of this book is not to give a detailed statistical account of Red Cross activities in Italy,—that may be found in the various Department Reports,—but rather to tell the American people who contributed so generously to the Red Cross funds the simple tale of what their dollars did in Italy. It is a great and inspiring record and one in which Americans may well take pride.

Resumen por el autor, George L. Streeter.

La emigración de la vesícula auditiva del renacuajo.

En el curso ordinario del desarrollo la vesícula auditiva del renacuajo experimenta una emigración definida, moviéndose desde el sitio en el cual se desprende de la piel a una posición más medial y dorsal, de tal modo que eventualmente viene a situarse muy cerca de la superficie lateral del cerebro posterior, con el apéndice endolinfático recubriendo el borde del delgado velo medular que forma el techo del cuarto ventrículo.

Esta particularidad, aparentemente, no es simplemente el resultado de un ajuste producido durante su desarrollo por la interacción de los procesos mecánicos de las estructuras adyacentes, sino que se debe, al menos parcialmente, a una tendencia autostática inherente a la vesícula misma, por medio de la cual mantiene y ajusta exactamente su posición durante el desarrollo con relación al cerebro y las estructuras que le rodean.

MIGRATION OF THE EAR VESICLE IN THE TADPOLE DURING NORMAL DEVELOPMENT

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ELEVEN FIGURES

In 1837 von Baer made the observation that the diaphragm is situated in the neck region in very young embryos, receiving its innervation from the cervical nerves, and that in the course of its development it acquires a more caudal position corresponding to the enlargement of the heart and lungs. This descent of the diaphragm was subsequently described in greater detail by Mall ('97). Uskow ('83) and Mall ('97) pointed out the marked shifting of position which the heart, lungs, liver, intestinal tract, and Wolffian bodies undergo during development in relation to each other and to the vertebral column. The migration of these organs in the embryo has given us the explanation of the peculiar course of their nerves of supply; for example, the inferior laryngeal, the vagus, the phrenic, and the splanchnic nerves. Kölliker ('61) showed that the shifting in position of the spinal cord produces an elongation of the spinal-nerve roots and the formation of the cauda equina. The influence of this factor in the formation of the filum terminale has been recently studied by Kunitomo ('18). It has been shown by Lewis ('01, '10) that such muscles as the trapezius and the eye muscles undergo considerable shifting in position between the time of their first appearance and the time when they have acquired their permanent attachments. Among others, Futamura ('06) has described the migration of the facial muscles and the consequent deflected distribution of the branches of the facial nerve. Within the central nervous system there are several instances where the

component nuclear masses exhibit a distinct migration in the course of their development (Streeter, '08; Kappers, '10). As a result of their disproportionate growth, the primary divisions of the brain shift into new positions relative to each other, and this is accompanied by an interesting adjustment on the part of the vascular drainage of these structures. Kohn ('07) and others have shown that the sympathetic ganglia undergo an extensive peripheral migration. When the places of origin of the thymus and thyroid glands were first discovered, it was recognized that these organs exhibit a conspicuous type of migration. Even in the case of the skeleton, it has been maintained (Rosenberg, '76) that the point of vertebral articulation of the pelvic girdle moves along the column into the lumbar territory during development, although this has not been adequately substantiated. There is, however, a very good example of topographical adjustment of bony structures in the case of the teeth.

To any one occupied with the study of organogenesis, the developmental alteration in topography that is everywhere in progress is very striking. In some cases it is obviously a matter of mechanical stress exerted by adjacent organs upon each other, the controlling factors being their relative increase in size and the relative resistance of their tissues; or it may be a matter of traction in connected organs. In other instances we find structures invading new territories by virtue of the direction of their growth, which is dependent on the fact that the proliferation and increase in size of their constituent cells are more active in one direction than in another. In some cases this is associated with a thinning out and disappearance (possibly dedifferentiation) of the opposite pole of the organ, resulting in its complete transposition. Such factors are easily understood and various combinations of them explain most of the instances of developmental topographical alteration in organs which we encounter. There are other cases, however, which are more obscure and in which the movement of the organs or structures during their development cannot be entirely explained by simple mechanical factors; in these the phenomenon resembles a true migration such as is seen in individual cells. For lack of a better explanation, we

must consider the possibility of the existence of some force, of the nature of a chemotaxis, interacting between these structures and their environment. It is in this group that we must place the ear vesicle which, during the course of normal development, exhibits a considerable change in position. It is to this that I would call attention here.

From several studies previously reported by the writer (Streeter, '07, '09, '14) and from a recent paper by Ogawa ('21), it is apparent that the determining factors in the posture of the membranous labyrinth involve something more than a passive development of the ear vesicle in the position in which it originally finds itself. It is clearly evident, moreover, that the final position of the labyrinth is not simply the result of an adjustment brought about during its development by the interaction of mechanical processes of the adjacent structures, but that it is due, in part at least, to an autostatic tendency inherent in the vesicle itself, by virtue of which it maintains and accurately adjusts its position during development with reference to the brain and the surrounding structures. When an early ear vesicle is experimentally rotated into an abnormal position, or transplanted in an abnormal position to the opposite side of the same specimen or to another specimen, it subsequently tends to correct its posture, and the final labyrinth, in spite of the previous displacement, is found to possess normal topographical relations.

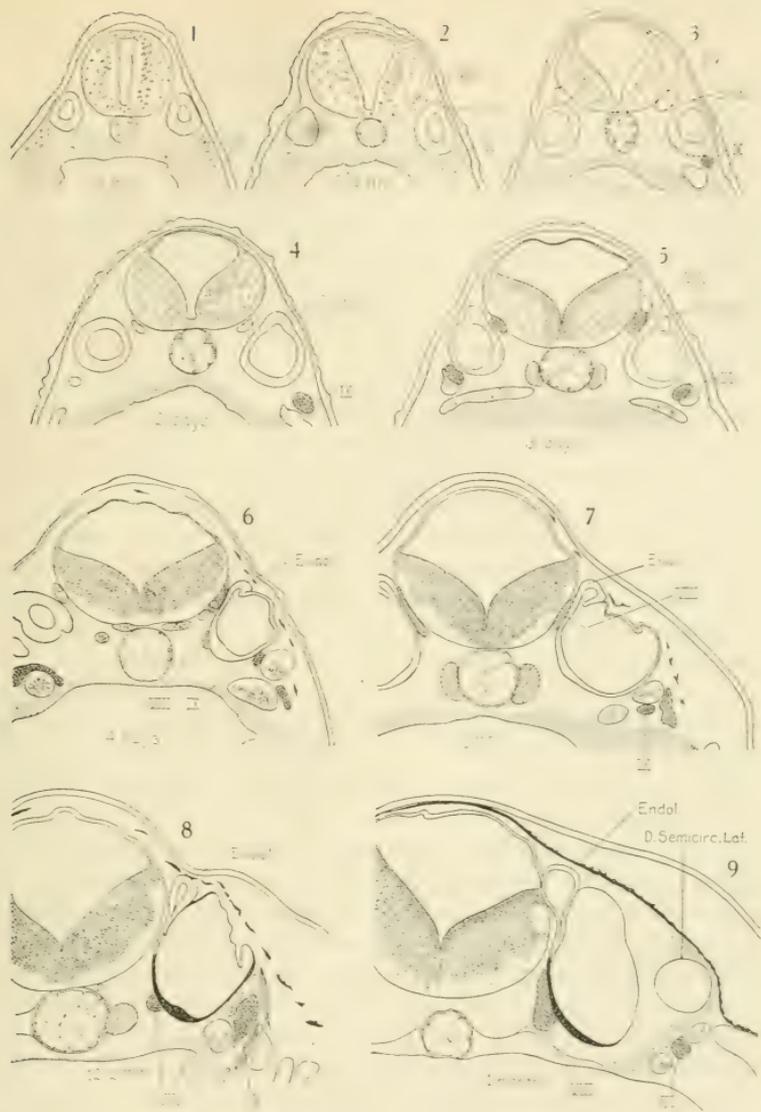
It is of interest to record that not only under artificial conditions, but also in the ordinary course of development, the ear vesicle of the tadpole undergoes a definite migration, moving from the point of its detachment from the skin to a more median and dorsal location, so that it eventually lies close against the side of the hindbrain with its endolymphatic appendage overlapping the margin of the thin medullary velum that forms the roof of the fourth ventricle.

If one prepares sections through the auditory region in a series of tadpoles covering the period between the premitotic stage and the end of the first month, the changing relations of the ear vesicle to the surrounding structures can be readily made out (figs. 1 to 9). These figures are made with the same

magnification and thus, by comparing them, it is possible to determine the actual increase in size, as well as the differentiation of the walls and the alteration in the position of the individual vesicles. In figures 7 and 8 the length of the specimens is given; in the remaining figures the age given is the length of time the specimen was allowed to develop after reaching the operating stage, i.e., when it has acquired a distinct tail bud and gill eminences, but has not yet exhibited any motor response to stimuli.

In the first stage shown (fig. 1) the relatively thin lateral wall of the ear vesicle lies tight against the ectoderm. The vesicle is separated from the thick endoderm and the brain tube by a narrow interval filled with mesenchyme which is beginning to show open spaces in the vicinity of the notochord, elsewhere being relatively compact and heavily laden with yolk. As yet there are no blood-vessels in this region and the acoustic nerve and ganglion are not clearly differentiated from the surrounding tissue. It will be noticed that the lateral plate of the brain tube lies in a vertical plane and the point at which the ventral nerve roots are to converge lies opposite the dorsal tip of the ear vesicle.

In the next stage (fig. 2) the ear vesicle remains in close contact with the ectoderm. The surrounding mesenchyme is assuming a reticular character and in it the primary blood-vessels can be recognized. The acoustic ganglion is distinctly marked off, being attached to the anteromedian surface of the vesicle and connected by a strand with the brain wall. The roof of the latter is thinning out and the lateral walls are undergoing eversion. In the third stage (fig. 3) the conditions are essentially the same, although the vesicle wall has undergone further differentiation, the mesenchyme is distinctly reticular, and there is further eversion of the brain wall. In the sections oral to the one selected for illustration, the acoustic-nerve ganglion is present. It can be seen that the ear vesicle at this time is widely separated from the brain and almost wholly ventral to it. The intervening mesenchyme is loose and would offer slight mechanical obstruction to the migration of the vesicle, and



Figs. 1 to 9 Sections showing the changes in the topographical relations of the ear vesicle of the tadpole during the period between the premitile stage and the end of the first month. In figures 1 to 6 and figure 9 the age given is the length of time the larva was allowed to develop after reaching the operating stage; i.e., tail bud and gill eminences present, but no motor response to stimuli exhibited. $\times 50$. *B.v.*, primitive blood-vessel plexus; *Endol.*, endolymphatic appendage; *VIII*, acoustic nerve ganglion; *IX*, glossopharyngeal nerve ganglion.

certainly the primary brain blood-vessel cannot be regarded as a serious obstruction, since we find that even a much more mature vascular system can readily accommodate itself to any movement of the surrounding organs. A brilliant example of this is seen in the case of the venous drainage of the fetal cerebrum. Migration, however, cannot occur so long as the vesicle adheres to the ectoderm. Its detachment therefrom becomes complete in the next two stages.

The stage illustrated in figure 4 is at the critical point where the vesicle is becoming detached coincident with an invasion of mesenchyme between it and the ectoderm. At the same time the brain shows further development of the roof of the fourth ventricle and continued eversion of its walls which tends to thrust it toward the ear vesicle. The vesicle itself is assuming a more dorsal position, as compared with the previous stage. The portion that is to form the endolymphatic appendage can be clearly recognized from five hours on; by the second day it is not only thicker than the rest of the wall of the vesicle, but also shows a beginning evagination and a distinct differentiation of its component cells. A conception of the shifting that is in progress can be obtained by the realization that the endolymphatic appendage of figure 4 will eventually overlap the rhombic lip of the brain wall.

By the third day (fig. 5) the upper half of the ear vesicle is above the level of the junction of the brain and notochord and is surrounded on all sides by reticular mesenchyme which should favor its migration. Its only attachment is that of the acoustic nerve ganglion which forms a massive strand firmly attached at one end to the brain wall and at the other to the anteromedial wall of the ear vesicle. From the differentiation of the mantle zone of the brain wall it can be seen that the point of attachment of the nerve corresponds closely to its permanent point of entrance and, tracing it peripherally to the vesicle wall, its fibers can be followed to the macular area. The size and character of the acoustic nerve might lead one to attribute to it a definite influence in any subsequent movement of the vesicle; but we know that the phrenic nerve exhibits no restraining influence in

the descent of the diaphragm and there is no evidence that the facial nerve exercises any guiding force in the migration of the musculature of the face. It is to be noted that there is still a relatively wide interval between the vesicle and the brain wall. As yet the mesenchyme shows no differentiation into skeletal framework, but on each side of the notochord can be seen the oral extension of the spinal musculature.

Up to the fourth day (fig. 6) there has been a gradual thinning of the main part of the wall of the ear vesicle, accompanied by an increase in the amount of the contained otic fluid, and at this time the first steps occur in the formation of the semicircular ducts. A little more than half of the vesicle is now above the level of the junction of the notochord and the brain. The vesicle and brain wall are more closely approximated, which may be explained in part by the further eversion and growth of the latter. On the other hand, there is a beginning differentiation of the subcutaneous tissues and pigment membrane producing an increase in the distance between the ear vesicle and the surface of the larva.

In larvae 9 mm. long (fig. 7) one finds the formation of the semicircular ducts well under way and at the same time the mesenchyme lateral to the ear vesicle is differentiated into a characteristic subcutaneous tissue, while that median to the vesicle shows a condensation into precartilaginous tissue. Spreading from the chordal area toward the ear vesicle and surrounding the brain can be seen arachnoidal spaces of a primitive type. Notwithstanding this more permanent type of environment, the dorsal migration of the vesicle is not yet complete, for in older stages almost the entire vesicle lies dorsal to the level of the chorda.

In larvae 12 mm. long the endolymphatic appendage and the dorsal crest of the vesicle have nearly reached the level of the rhombic lip, and at the same time the lowest point of the vesicle lies opposite the level of the center of the notochord. The dorsal extension at this time is due in part to the direction of the growth, associated with the formation of the anterior and posterior semicircular ducts and the increase in the length of the endolymphatic appendage.

At one month the ear vesicle is completely differentiated into a membranous labyrinth with three semicircular ducts and a characteristic macular area to which is attached the ganglion and its peripheral nerve terminations. A characteristic endolymphatic appendage is present, consisting of a relatively large sac connected by a slender duct with the vestibular portion of the labyrinth. As can be seen in figure 9, the sac now lies in close contact with the thin roof of the fourth ventricle. The labyrinth lies wholly dorsal to the midlevel of the notochord and is secured in this position by the mesenchymal otic capsule,

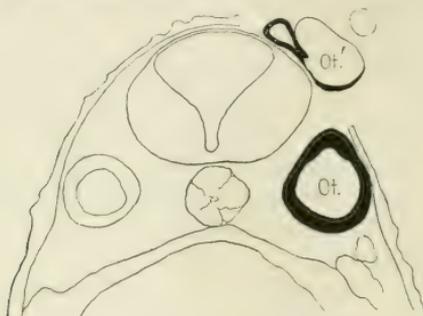


Fig. 10 Diagram showing the migration of the ear vesicle relative to the brain wall from the position it occupies at the end of the second day (*Ol.*) to the position it attains as a differentiated labyrinth at the end of the first month (*Ol'*), the brain wall being represented as stationary.

consisting of precartilaginous tissue, portions of which are already differentiated into typical cartilage cells surrounded by a homogeneous matrix. With this stage the essential relations of the labyrinth may be regarded as established; the subsequent minor changes in its topography are those determined by the mechanical factors of its own further growth and the further growth and differentiation of the surrounding structures.

From the foregoing comparison of the individual stages it is clear that the ear vesicle shifts its position relative to the brain wall to the extent diagrammatically shown in figure 10. Whereas at the end of the second day the vesicle lies ventral to and apart

from the brain, at the end of the first month it is situated close against the lateral brain wall with its endolymphatic appendage overlapping the rhombic lip. In the figure there has been no account taken of the lateral movement of the brain wall, and therefore to that extent the path of migration of the ear vesicle is exaggerated. Its dorsal migration relative to the notochord, the ectoderm, and the everted brain wall may be represented as in figure 11, which shows more accurately than figure 10 the extent of its change of position relative to the whole environment. Although the normal migration of the ear vesicle is not so marked

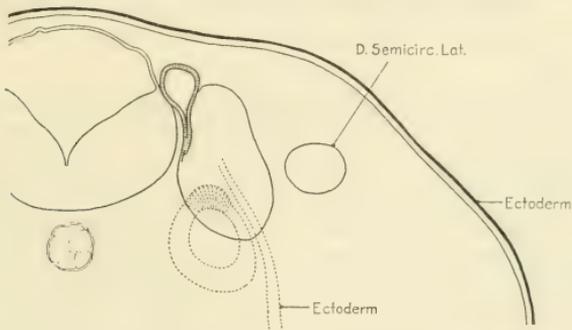


Fig. 11 Superimposed sections of the ear region of a tadpole of the nineteenth hour (dotted) and of another at the end of the first month, enlarged so that the brain is the same size in both cases, the two being fitted so as to exactly coincide.

as that of the thymus and many other organs, nevertheless that phenomenon unquestionably occurs. To some extent the mechanical forces of growth of the concerned parts can be recognized as influencing the migration; no single one of these factors, however, or no combination of them would appear to adequately explain it.

The detachment of the vesicle from the skin is readily explained by the differentiation of the subcutaneous tissues and the formation of the pigment membrane. This begins to take place about the second day. By the fourth day the elements of the pigment membrane make their appearance, and in tadpoles 12 mm. long there is a relatively complete membrane separating the vesicle

from the loose tissue underlying the ectoderm. This differentiation releases the vesicle from its firm attachment to the ectoderm, but it does not in any way favor its dorsal migration.

The change in the relative position of the ear vesicle and the brain wall is in part accounted for by the direction of growth of the latter, which undergoes an eversion whereby it is thrust ventralward and lateralward toward the vesicle. The maximum effect of this eversion is reached at the end of the fourth day, but at this time, in addition to the close approximation of the brain wall and the ear vesicle, a dorsal shifting of the latter has occurred relative to the level of the notochord, a fact which can hardly be explained by the change in position of the lateral brain plate.

As to this dorsal migration of the vesicle as a whole, which takes place gradually throughout the first month, one should consider the possibility of its being due to the direction of growth of the vesicle; i. e., that the dorsal portions of the vesicle may perhaps grow more rapidly than the ventral portions. In the case of the endolymphatic appendage, the direction of growth may very well constitute a factor in the attainment of its final position. The dorsal growth of the sac and the elongation of its duct would favor its dorsal shifting. Aside from the slender endolymphatic duct, however, there appears to be nothing to prevent the sac from occasionally going astray orally or caudally, where the tissues would offer little obstruction to its extension in these directions. That it never does so forces one to postulate the existence of some form of determinative attraction between the endolymphatic sac and the medullary roof to which it later invariably becomes intimately attached.

There is no evidence that the acoustic nerve ganglion plays any considerable part in the way of a guiding or traction force. The nerve can be recognized at the fifth hour, connecting the vesicle with the brain wall, but when it is experimentally detached, as in the transplantation of a vesicle from one tadpole to another, the severing does not interfere with a correct adjustment of the posture of the vesicle. This corresponds to our experience with other organs, in which the nerves do not act as a check or show

any evidence of influencing the migration of the organs in any way. The surrounding mesenchyme and primitive blood vessels can also be dismissed as factors.

The cartilaginous skull does not make its appearance until the final relations of the labyrinth to its environment are essentially established, that is, toward the end of the first month, and therefore cannot play a primary part in the migration of the vesicle. However, after the firm otic capsule becomes differentiated the latter must absolutely control those further alterations in the posture of the contained labyrinth which are associated with the final changes in the form of the base of the skull.

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Resumen por los autores, E. L. y E. R. Clark.

El carácter de los linfáticos en el edema experimental.

Los autores han producido edema en el renacuajo mediante: (1) Extirpación del pronefros; (2) extirpación del esbozo del corazón; (3) impidiendo el desarrollo de la musculatura del corazón linfático, y (4) en conexión con la inflamación aséptica.

1. En los individuos sin pronefros, el líquido plasmático se acumula tan solo en la cavidad abdominal. La circulación sanguínea se hace más difícil y el desarrollo de los vasos sanguíneos se retarda. Los linfáticos se desarrollan, y en apariencia funcionan normalmente.
2. Cuando se extirpa el corazón el edema está limitado a las cavidades del cuerpo. Los capilares linfáticos de la cola aparecen un poco más grandes que lo normal.
3. En renacuajos desprovistos de corazón linfático contractil grandes cantidades de líquido se acumulan en los senos cefálicos, y eventualmente en el tejido subcutáneo del cuerpo y cola.
4. En el edema inflamatorio los capilares linfáticos de la región afectada se distienden.

En embriones de pollo, los autores impidieron el desarrollo del corazón linfático cortando la cola en los embriones de tres días. A los siete días los embriones están edematosos. La inyección demuestra la presencia de linfáticos distendidos en forma de saco, en vez de aparecer como los conductos más pequeños, presentes en los embriones normales.

Conclusiones:

1. Los capilares linfáticos se desarrollan normalmente, absorbiendo linfa, sin circulación sanguínea o con circulación defectuosa.
2. Cuando se impide la salida del líquido que ocupa los linfáticos estos se distienden.
3. La acumulación de fluido en los espacios de los tejidos está asociada con una dilatación de los capilares linfáticos. Este resultado se opone a la idea generalmente mantenida por los patólogos, de que los capilares linfáticos se contraen en el edema.

THE CHARACTER OF THE LYMPHATICS IN EXPERIMENTAL EDEMA

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FIVE FIGURES

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INTRODUCTION

Abnormal collections of fluid in the tissue spaces and serous cavities may be caused by a disturbance in any of the factors concerned with the normal formation transudation and absorption of lymph in the animal organism. The causes are probably different in the different types of edema and a number of causes may be involved simultaneously (Wells, '18). Although the lymphatic system is known to be intimately concerned with the normal absorption of fluid, the relation of the lymphatics to edema has never been thoroughly studied. It is well known that edema of a limb may be caused by blocking of the main lymph channels or lymph glands draining the limb, but this type is of relatively rare occurrence in adult warm-blooded animals, owing to the ability of the veins to take over a large share of the absorptive function of the lymphatics.

Smith and Birmingham ('89) have reported a case of edema of the foetus in which they claim that there was a total absence of lymphatic system.

The reaction of the lymphatic capillaries to the presence of an increased amount of tissue fluid, found in cases of generalized edema of the subcutaneous tissues, is not well understood. According to Adami ('09), the delicate walls of the lymphatic capillaries collapse under the increased pressure of the surrounding fluid.

We have attempted to produce edema experimentally by several methods with the particular object of studying the effect of this condition upon the lymphatic capillaries. Tadpoles were used for these experiments, because of the possibility of watching the lymph capillaries in the living, in the transparent-fin expansion. Some experiments were also performed upon chick embryos.

EXPERIMENTAL EDEMA IN AMPHIBIAN LARVAE

Two species of frog larvae were used—*Rana pipiens* and *Rana catesbiana*. The operations were performed under the binocular microscope, using chloretone anaesthesia. Small glass needles were used for most of the dissections and iridectomy scissors for the removal of the anlage of the blood heart.

The lymphatic vessels of the edematous specimens and of normal larvae of the same ages were injected with india ink. The capillaries of the transparent fins were studied in the living in the observation chamber previously described (E. R. Clark, '12).

The following methods were tried for producing edema in tadpoles: 1) removal of the pronephros; 2) removal of the anlage of the blood heart; 3) removal of somites to prevent the development of the lymph heart; 4) injection of drops of croton oil to produce inflammation (E. R. and E. L. Clark, '20); 5) acetic acid.

1. Removal of the pronephros

This experiment was performed upon a number of tadpoles, during the spring and summer of 1915. Since the general re-

sults of this operation have been described by Howland ('16) and Swingle ('19), it is unnecessary to give a detailed description. Edema developed on the day following the operation and was of the type of ascites, fluid collecting only in the abdominal cavity. During the succeeding days, the development of the tadpoles was greatly retarded, the gills remained external for a longer period than in the controls, the coils of the intestine were fewer, the head and eyes smaller, the tail remained much shorter and more pigmented, and the heart beat sluggishly.

Microscopic observation of the transparent tails of such larvae showed a more sluggish circulation and fewer blood capillaries than in the normal specimens. However, the lymph hearts of the larvae with pronephros removed beat as strongly as did those of the controls, and even more frequently, and the lymphatic vessels of the tail had extended beyond the blood-vessels and were normal in appearance.

2. Removal of the blood heart

The operation for the removal of the heart in tadpoles has been described by Knowler ('07) and the effect upon blood-vessels and lymphatics by E. R. Clark ('18). Embryos operated on in this manner soon become edematous—the collection of fluid being confined to the body cavities, the tail remaining small and pigmented.

Although the development of the blood-vessels is greatly retarded with the heart absent, that of the lymphatics is not. The lymph hearts are larger and beat more strongly than do those of normal larvae of the same age and there is an active movement of fluid inside the lymph-vessels as demonstrated by the occasional presence of blood-cells within the lymphatics, which were observed to move along with the current.

In studying the lymphatic capillaries of the tail it was found that these vessels develop normally and often extend beyond the blood capillaries, although in normal embryos of the same age and species, the blood-vessels of this region grow out well in advance of the lymphatics. Moreover, the lymphatics are wider than in normal animals (E. R. Clark, '18).

This experiment showed that lymph continues to pass into the lymphatic capillaries in the absence of the heartbeat and blood circulation and that the growth and absorptive power of lymphatics are not dependent upon the blood pressure.

3. Operation to prevent the development of the lymph-heart musculature

For this experiment the somites dorsoposterior to the pronephros were removed on both sides of the larva. In the majority of cases this effectively prevented the development of the pulsating lymph hearts. Such tadpoles developed normally and were practically indistinguishable from the controls for the first four or five days after the operation. On the sixth or seventh day after the operation, edema of the head region of these embryos makes its appearance. This enlargement was noticed invariably on the same day at which the first pulsation of the lymph hearts was observed in normal tadpoles of the same age. During the following days the sinuses of the head became still more distended, while those at the sides of the body also enlarged and finally, ten to fourteen days after the operation, the tail became edematous. In contrast to the larvae deprived of their pronephroi or blood hearts, these tadpoles did not contain an excessive amount of fluid in their abdominal cavities.

The tadpoles without beating lymph hearts lived for three weeks after the operation—the longest period of survival being twenty-six days. During the first two weeks after the operation the tadpoles were as large as the controls, and practically normal except for the edema. During the last week of life, however, the blood circulation became impaired and often ceased altogether.

In the larvae without lymph hearts, the lymph-vessels were studied by injection of india ink into the main dorsal and ventral lymph-vessels of the tail. The dorsal vessel normally empties into the right lymph heart and the ventral vessel into the left. In the case of the operated tadpoles, the dorsal vessel emptied into a large sinus on the right side and the ventral vessel into a

similar sinus on the left side. These tail vessels together with their branches were found to be wider than those of the controls and the pressure inside them was found to be high, as shown by the effort required to fill them with the injection material.

Microscopic examination of the transparent tails of the tadpoles without lymph hearts showed no difference from the normal during the first week after operation. At the end of ten or twelve days the tail begins to show evidences of edema particularly at the base. During the next week these changes become visible throughout the whole tail. The actual increase in the thickness of the tail was measured by means of the micrometer screw of the fine adjustment, by focusing on a certain point on the surface of the fin and turning the screw until the opposite surface came into focus, and meanwhile counting the divisions on the screw. The edema is further detected by the changed appearance of the tissue in the fin; the whole region becomes clearer and the connective-tissue cells are more widely separated than in the normal tadpoles. The cells themselves do not enlarge.

Associated with this increase in fluid present in the connective-tissue spaces, we invariably found an enlargement of the lymphatic capillaries. The vessels near the base of the tail are first affected and later the more posterior ones enlarge also. Figures 1, 2, and 3 show the difference in size of the lymphatic capillaries in these larvae in contrast to that of the blood capillaries of the same specimens and to that of the lymph capillaries of normal tadpoles.

In addition to change in caliber, lymphatics of these tadpoles with generalized edema also show changes in contour—the irregular outline with abundant fine processes characteristic of the normal lymph capillary is lost and the endothelium becomes smoother and thinner.

4. Inflammatory edema

The cell reactions which take place after injection of minute globules of croton oil into the tail fins of amphibian larvae, have been described in a recent publication (E. R. and E. L. Clark, '20).

In a number of instances a localized edema was observed at the site of injury. The increase in the amount of fluid in the region was easily detected by the increased transparency of the region and by the greater distance between the connective-tissue cells. The increase in the thickness was measured by means of the micrometer fine-adjustment screw. The lymphatic capillary sprouts of the edematous region are always wider than similar vessels of neighboring areas: in fact, the simultaneous enlargement of

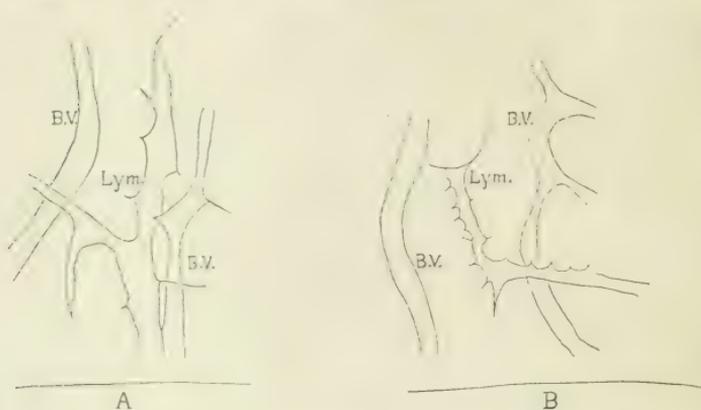


Fig. 1 A. Drawing of vessels from the ventral fin of a tadpole ten days after operation for removal of the lymph hearts. *Lym.*, lymphatic; *B.V.*, blood-vessel. Thickness of the tail at this point, 310μ . B. Drawing of the same region in the ventral fin of a normal tadpole of the same age. Thickness of the tail, 180μ . Note difference in the size of the lymphatic in the two specimens. *B.V.*, blood-vessel; *Lym.*, lymphatic. $\times 175$.

these capillaries was found to coincide precisely with the increase in intercellular fluid (fig. 15, in article by E. R. and E. L. Clark, *The American Journal of Anatomy*, May, 1920). This enlargement of the lymphatics is characterized by a widening of the lumen of the lymphatics, while the endothelial wall becomes thinner and smoother. These lymphatics regain their normal caliber and contour with a return of the region to its normal thickness. The blood capillaries of such regions show no such changes.

5. Experiments with acetic acid

These experiments were suggested by the work of Martin Fischer (15) which connects the development of edema with the presence of an acidosis in the tissues. Tadpoles were placed in

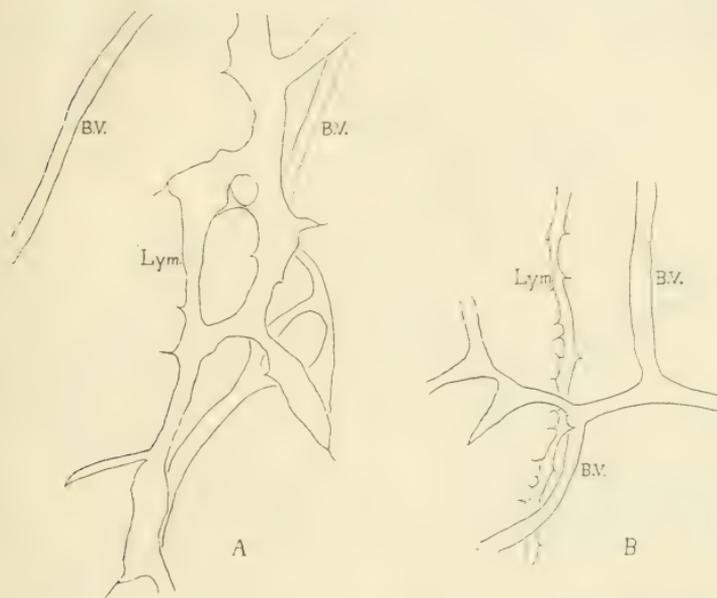


Fig. 2 A. Drawing of vessels from the dorsal fin of a tadpole fourteen days after operation for removal of the lymph hearts. Thickness of the tail at this point, 340μ . B. Vessels from the same region of a normal tadpole of the same age. Thickness of the tail, 170μ . This shows still greater enlargement of the lymphatic capillary than in figure 1, A. B.V., blood-vessel; Lym., lymphatic. $\times 175$.

varying strengths of acetic acid, with the object of producing edematous tadpoles. The results of these experiments were as follows:

All tadpoles in strengths of acetic acid of 1 to 2000 or stronger were dead at the end of an hour and a half.

Tadpoles left overnight in strengths of acetic acid from 1 to 5000 up to 1 to 15,000 were all dead on the following morning.

Tadpoles in 1 to 20,000 and 1 to 30,000 survived, and at the end of a week they were as large, active and well developed as the controls and showed no signs of edema.

Tadpoles placed in 1 to 18,000 and 1 to 19,000 died within twenty-four hours.

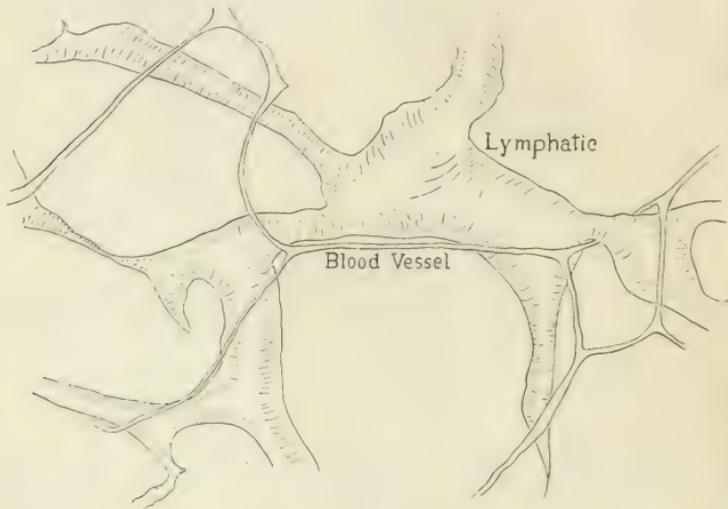


Fig. 3 Drawing of the vessels of the posterior portion of the abdominal wall of an edematous tadpole fifteen days after operation for the removal of the lymph hearts. The lymphatics are greatly distended while the blood-vessels are narrower than normal. $\times 158$.

These experiments were negative in regard to the production of edema in tadpoles by the use of acetic acid in the surrounding fluid, since all specimens died when the acid was stronger than 1 to 20,000, while in this and in weaker strengths of acid no edema developed. Obviously, these results do not necessarily have any bearing whatever on the theory that edema may be caused by acidosis, since the presence of a trace of acid in the surrounding medium may have had no effect on the acidity of the body fluids.

EXPERIMENTAL EDEMA IN CHICK EMBRYOS

We attempted unsuccessfully to produce edematous chick embryos by chemical means—acetic acid was placed in an open dish in the incubator and alcohol also was tried without success. Only one form of edema was produced in chick embryos—that which resulted from the removal of the posterior lymph hearts. The development of the lymph heart is prevented by cutting off the tail rudiment in embryos of two to three days' incubation. This operation and the early development of the lymphatics in such embryos have been described elsewhere (E. R. and E. L. Clark, '19).

It was shown in a former publication (E. L. Clark, '15) that the lymph flow in the early superficial lymphatics of chick embryos is dependent to a considerable extent upon the pulsation of the posterior lymph hearts. It was shown that the commencement of lymph heart pulsations, in chicks of $6\frac{1}{2}$ days, is the factor which instigates the lymph flow in the posterior half of the embryo. The area drained by the lymph heart increases until, in embryos of seven to eight days, the direction of the entire superficial lymph flow is posterior through the lymph hearts into the veins of the tail. Associated with the establishment of the lymph flow in the superficial lymphatic plexus, channels develop in the exact places where the movement of lymph had been demonstrated in the living chick by the injection of india ink into the superficial lymph capillaries. In addition to the formation of these ducts, the former investigation showed that the lymphatics in later stages enlarge to form sacs at points where two conflicting pressures occur. In this later stage, eight to nine days, the tissue is very loose—so much so, in fact, that it might almost be called edematous, were it not for the fact of its normal occurrence. At this later stage the lymph hearts are chiefly concerned with the flow of lymph from the allantois.

In the former publication a case was described in which an embryo of seven days possessed a stunted tail with a small feebly beating lymph heart. This embryo was edematous and the lymphatics of the pelvis which drained into the lymph heart were

large and distended, greatly resembling the sacs normally present in this region in chicks of eight and one-half days.

In the operated tailless chicks of six and one-half days, the stage at which the lymph heart begins to beat in normal chicks, the anterior lymphatics are normal as regards appearance, development of the main duct, and direction of lymph flow. The posterior half of the body, however, is markedly edematous in these chicks and the channels normally present over the pelvis



Fig. 4 Drawing of an embryo chick of seven days, in which the tail containing rudiment of the lymph hearts had been removed at the three-day stage. Compare character of the lymphatic plexus, injected with india ink, with that of a normal chick of the same age (fig. 5). The absence of definite channels in the posterior half of the body and the enlargement of the lymphatic capillaries over the pelvis are particularly noticeable. $\times 4.67$.

are absent and in their place is an irregular plexus of the primitive type, the vessels composing which are much larger than usual.

In chicks of seven to seven and one-half days, the stage at which the lymph flow of the entire superficial lymphatic system is normally influenced by the beating of the lymph heart, the chicks were always found to be edematous after the operation for the removal of the lymph heart. The edema is noticeable to the naked eye; it is evident from the greater distance necessary to plunge the injecting cannula before reaching the superficial lymphatics, and also from the greater spaces present between the connective-tissue cells in microscopic sections of such embryos.

In these chicks, channels over the anterior body wall and pelvis are absent and in their place an irregular plexus is present, many of the vessels of which are greatly enlarged and sac-like in appearance (fig. 4). It is evident from the difficulty encountered in



Fig. 5 Drawing of a normal chick of six days and twenty-two hours, showing injected superficial lymphatics with newly formed lymph ducts and lymph heart (L.H.). $\times 5$. (Copied from Clark, E. L., '15, fig. 4.)

injecting these vessels over the pelvis that the fluid in them is under high pressure.

In older stages, that is, in chicks of eight and one-half days and older, the tailless embryos are not noticeably more edematous than normal specimens of the same age. Injections of the superficial lymphatics show in most cases that the lymphatics from the two sides have anastomosed over the stump. Otherwise they

possess the same channels and superficial sacs as the normal specimens. It will be remembered that in normal chicks of eight and one-half days and over, the lymph heart is only slightly concerned with the lymph flow in the superficial lymphatics, being chiefly concerned with the flow of lymph from the allantois. It is interesting to find in these operated chicks of eight to nine days that the lymphatics of the allantois are so distended as to render them easily visible to the naked eye without injection.

These operations on chicks have added more evidence to that already reported with regard to the importance of the lymph heart in instigating and influencing the early flow of lymph in the superficial lymphatic plexus and in determining the formation of ducts in the posterior part of the body. They also show that when the lymph flow is interfered with in these early stages, by removal of the lymph heart, embryos become edematous, the development of ducts is interfered with, while the vessels of the superficial lymphatic plexus enlarge greatly until they become even larger and more distended than the sacs of older chicks.

DISCUSSION AND CONCLUSION

The ease with which experimental edema is produced in amphibian larvae by any interference in the flow of fluid in the lymphatics is undoubtedly due to the importance of the lymphatic system of the lower vertebrates in connection with the absorption of water. Maxwell ('13) has found that a relatively enormous quantity of water passes through the frog's skin, and Moore ('15) has shown that a large part of this absorbed water is carried off by the lymphatics. In the lower vertebrates in which there is an active absorption of water through the skin, lymph hearts which assist in maintaining the flow of lymph are always present. In birds, the two lymph hearts, one on each side of the tail, function during embryonic life, but usually atrophy at the time of hatching (exceptions to this rule being water birds and the ostrich and cassowary). In the higher vertebrates—mammals and most adult birds, in which the absorption of water through the skin is practically absent—the importance of the lymphatic system as a drainage system is evidently diminished. Mam-

mals do not possess lymph hearts, but with the appearance of lymph glands the lymphatic system takes on new and important functions not found in lower vertebrates.

The fact that lymph hearts are present in all birds during development, which takes place in a fluid medium, suggests the possibility that there may be an absorption of fluid through the skin of the higher vertebrates during their embryonic life with a resultant increase in absorption of fluid by the lymphatics. In a former paper (E. L. Clark, '15) it was shown that in the chick embryo lymph sacs develop at a stage in which the pressure inside the lymphatic vessels is high, probably owing to increased absorption and the outlet into the veins interfered with, and that such sacs always develop at points where conflicting pressures occur. It is possible that the early development of lymph sacs in mammalian embryos at points where the lymphatic system communicates with the veins may be due to the fact that this actively functioning drainage system has no pulsating lymph heart to assist in the flow of fluid from the tissues.

Moreover, the present experiments, which have shown the important effects upon the absorption of fluid from the tissues resulting from an interference with the drainage of fluid by the lymphatics, in tadpoles and in chick embryos, suggest the possibility that edema of the human embryo may also be caused by a blockage of the main lymph channels, especially of the thoracic duct.¹

¹Smith and Birmingham ('89) assert that in the edematous foetus which they studied the lymphatic system was entirely absent. However, the illustration which they give (a low-power drawing of a microscopic section) contains certain structures which are unquestionably lymphatics, and not 'spaces' as they are labeled. Microscopic studies of edematous tissues (Marchand, '11; E. R. Clark, '16, and this article) have shown that fluid in subcutaneous tissues does not collect in 'lakelets,' but that it is evenly distributed, separating the cells in a uniform manner. The more probable explanation for this interesting case would appear to be that instead of being absent, the lymphatic system was blocked at some important point—perhaps at the outlet of the thoracic duct—and that there had then occurred a generalized edema of the embryo and a simultaneous distention of the lymphatics including the finest capillaries. It is interesting to see that in this case the 'spaces' in the mesenchyme are much larger than the blood capillaries.

The experiments reported here have yielded the following results in regard to the relation of the lymphatics to experimental edema:

With the removal of the pronephros or the blood heart in amphibian larvae and the consequent abnormal collection of fluid in the serous cavities, the lymphatics continue to develop and to function in a normal manner. Their development and function are not interfered with even by the absence of circulation in the blood vascular system.

Generalized edema of the embryo may readily be produced by mechanical interference with the outflow of fluid from the lymphatics (removal of the lymph hearts in tadpoles and in chick embryos).

In such cases of generalized edema the lymphatics invariably enlarge and the delicate lymph capillaries do not collapse with the increased pressure outside the vessels, but, on the contrary, they continue to absorb fluid until they become greatly distended.

In cases of localized edema, in areas where an inflammation has been produced, the lymphatic capillaries of the edematous region respond to an increase in the fluid outside by enlarging at the tips, and they resume their normal size with the disappearance of the edema.

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Resumen por el autor, S. E. Whitnall.

Algunos músculos anormales de la órbita.

El autor describe un músculo elevador de los párpados superior que presenta dos anomalías comunes de los fascículos que pasan: (a) A la polea del músculo oblicuo superior (músculo tensor de la troclea, de Budge), y (b) A la glándula lacrimal; y en adición, (c) existe una banda transversa y entrecruzada parcialmente (músculo orbitario transverso). El origen del músculo oblicuo inferior del globo ocular fué examinado en cien órbitas, hallando el autor un desplazamiento lateral de su posición descrita como normal, (inmediatamente adyacente al borde de la incisura lacrimal u orificio superior del canal naso-lacrimal) en muchos casos, y en el 14 por ciento de los casos estaba separado un cuarto de pulgada, próximamente. En un caso, cuando la distancia era 7 mm. la inserción ocular fué examinada, hallando el autor que estaba situada más arriba de lo normal. Otras anomalías observadas son las de los músculos rectos, mencionadas en la literatura, junto con ejemplos de conexiones entre los músculos, encontradas por el autor en una serie de disecciones.

SOME ABNORMAL MUSCLES OF THE ORBIT

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TWO FIGURES

The following instances of abnormal muscles were met within a series of dissections of the orbit.

LEVATOR PALPEBRAE SUPERIORIS

There are three variations from the normal in this instance which was found in the left orbit of an adult female cadaver, of which the other orbit was not available for dissection.

1. The most striking feature is the presence of a band of muscle fibers, 2 mm. broad, passing transversely across the anterior part of the orbit and interwoven to a certain extent with the fibers of the levator palpebrae superioris. The extremities of the band curve slightly backward to be attached to the periosteum of the medial and lateral walls at about the junction of their anterior and middle thirds.

2. From the medial margin of the levator itself a well-defined slip is separated off and passes in the direction of the trochlea or pulley of the tendon of the superior oblique muscle. This slip loses its fleshy character before it reaches the trochlea, being continued on by connective-tissue strands.

3. From the lateral margin of the levator a well-marked offshoot passes both to the orbital wall and also to the lacrimal gland. In two other cases I have seen similar fasciculi passing to the lacrimal gland. They are attached to the connective tissue which forms the so-called capsule at the posteromedial region of the gland, and traction upon the levator draws the gland slightly backward. Microscopical examination showed all these fibers to be cross-striped.

There have been described two abnormalities of the levator of the upper eyelid:

a. Where the muscle presents an offshoot from its medial border which passes to the pulley of the superior oblique muscle, replacing or reinforcing the normal fascial expansion of its sheath to that point. This muscular slip is the *tensor trochleae* of Budge ('59), and is identical, according to Macalister, with muscles described by Vesalius, Molinetti, Kolmus, Sandifort, and with

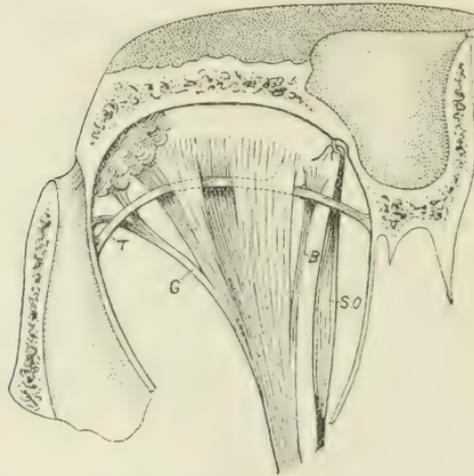


Fig. 1 Left orbit dissected from above to show an abnormal levator palpebrae superioris, showing *T.*, a transversus orbitis; *B.*, a gracillimus or tensor trochlear or comes obliqui superioris, as it is variously termed; *G.*, a slip passing to the lacrimal gland; *S.O.*, is the superior oblique muscle.

the *comes oblique superioris* of Albinus, and *gracillimus orbitis* of Bochkalek ('68). According to Budge, it is found in 10 per cent of cases, but in the writer's experience is much more rarely found in a well-defined state. In one preparation there were present two long muscle bundles, arising in common with the levator and ending anteriorly, one upon the fascia bulbi between the superior oblique and the globe, the other on the orbital margin beneath the pulley; the nerve supply came from the fourth nerve; the superior oblique was broader than usual. Ledouble

has found supernumerary fasciculi accompanying the reflected tendon of the superior oblique, and has further recorded a case where the direct or fleshy part of this muscle was absent, the reflected or normally tendinous part being muscular and arising from the site of the pulley, recalling the type found in non-mammalian vertebrates.

b. The *musculus transversus orbitis*, described by Bochdalek in 1868 as a muscle passing from the anterior and upper part of the os planum of the ethmoidal bone across the upper part of the orbit to its lateral wall. It consists at its origin of small tendinous bands enlarging to fleshy bundles which give off various attachments to neighboring fascia and especially to the levator palpebrae superioris with which it is closely connected; in fact, when the transversus orbitis is small it practically forms part of the levator. Macalister and Ledouble consider it as being a backwardly displaced slip of the orbicularis palpebrarum. This transversus orbitis is sometimes confused with the gracillimus, as, for example, by Howe ('07).

Perna ('05) described an 'abnormal transverse muscle' of the orbital cavity in man, which appears to be a transversus orbitis. He differs from Macalister as to the real origin of the muscle, considering it to represent the remains of the primitive muscular membrane which surrounds the organ of vision in lower vertebrates and which persists longest in phylogeny where the bony orbit is least complete and of least protection; he considers the levator itself to have the same origin, differentiated later for the special function of palpebral movement. An objection to this view is that the orbital periosteum, the periorbita, with its vestige of involuntary musculature found in the infra-orbital fissure in man and much more largely developed in certain lower animals, may be considered on sounder morphological grounds to be the representative of this primitive orbital cavity (Motais, '87; Groyer, '03). It may also be pointed out that the frontal nerve is lying between the planes represented by these two structures—the levator and the periorbita.

Neither would this anomaly appear to be derived from the peribulbar involuntary musculature described by Landström

('07) and recently re-investigated by Hesser ('13), since the fibers are of a different nature and lie on a much more superficial plane.

As regards the view of Macalister that the abnormality is formed by a portion of the orbicularis oculi, it is difficult to see how a portion of the superficial facial muscle sheet comes to be displaced to so deep a plane, posterior to the tarsal plates and septum orbitale, the latter of which is generally regarded as forming the anterior boundary of the orbital cavity, and why such fasciculi are so intimately connected with the levator.

A third explanation which might be considered is that the transversely disposed muscle fibers replace that thickened part of the superficial fascial sheath of the levator which runs in the same direction across the orbit and presents somewhat similar attachments and, it has been suggested, may act as a check ligament to the action of the muscle (Whitnall, '10). This view is supported by the fact that in the present instance the fascial sheath was but little developed and formed no transverse band such as is normally present. The difficulty here is to reconcile the fact that while it is not uncommon to find the reappearance of muscle fibers in connective tissue which has replaced muscle, as, for example, in the case of the panniculus carnosus, yet pre-existing connective tissue tends under strain rather to become condensed and developed into a definite ligament than to be replaced by muscle, as instanced by the appearance of definite ligaments in certain portions of the capsules of joints. On these grounds Perna's view is borne out by the numerous offshoots of the levator which may appear—it may be the remains of a much larger muscular sheet, but it is not homologous with the primitive membranous orbit as represented by the periorbita.

The present instance of an abnormal levator palpebrae superioris muscle is of interest in that it shows, though perhaps feebly developed, the two variations commonly described, the *tensor trochlea* and the *transversus orbitis*, and in addition an offshoot passing to the lacrimal gland. The latter could have a much more effective action as a *retractor glandulae lacrimalis* than the medial offshoots as a *tensor trochleae*, since the gland is fairly movable, the trochlea only slightly so.

MUSCULUS OBLIQUUS INFERIOR

The inferior oblique muscle of the eyeball is stated to arise from the anteromedial part of the floor of the orbit, just within the margin and immediately adjacent to the opening of the nasolacrimal canal (*incisura lacrimalis*). The site may be marked by a small oval impression and often the margin of the orbit is slightly lipped in this region. So close is the origin to the edge of the canal that occasionally fibers of the muscle are found to spring from the periosteal covering (*lacrimal fascia*) of the lacrimal sac at its base (fig. 2).

Since several cases were found by the writer where the origin was displaced laterally some distance from this normal site, it appeared worth while examining a series of orbits. Out of 100 orbits dissected the distance of the origin from the edge of the nasolacrimal canal was as follows:

Adjacent (0 to 1 mm. away).....	in 45 cases
2 mm. distant.....	in 14 cases
3 mm. distant.....	in 19 cases
4 mm. distant.....	in 8 cases
5 mm. distant.....	in 6 cases
6 mm. distant.....	in 4 cases
7 mm. distant.....	in 4 cases
Total.....	100

Approximately, therefore, the origin lay a quarter of an inch (5 to 7 mm.) lateral to the normal site in 14 per cent. the displacement was more commonly found in left orbits.

In both orbits of one subject (female) the origin of the muscle was found situated 7 mm. away from the orifice of the canal, and the insertion of the muscle onto the right eyeball was specially examined (fig. 3). The breadth of the insertion was 8 mm. (usually 9.9 mm.) and was placed above, instead of below, the horizontal meridian; its nearest point was 5 mm. from the optic nerve—a little less than usual; its central point was 9 mm. (usually 11 mm.) distant from a corresponding point on the line of insertion of the superior oblique muscle. The total length of the muscle was 37 mm., which appears normal. In two other cases

of similar origins examined the most marked differences of insertion were again in a higher position on the eyeball, though it should be added that, according to Howe, the ocular insertions of the inferior oblique are more variable than any of the other muscles. The length of the muscles was normal.

As regards the possible effect of such a laterally displaced origin on the action of the muscle, it is first to be noted that, since



Fig. 2 Diagram to show normal position of origin of the inferior oblique muscle and its insertion onto the eyeball.

the attachment is behind the equator of the globe, contraction of the muscle will tend to draw the latter forward, and the more directly the origin is situated in front of the eyeball, at a greater advantage can the muscle so act. In the second place, the effect of the displacement is to bring the origin nearer the insertion, and so the length of the muscle would be decreased. Since the length of a muscle, in which the fibers are arranged parallel to the long axis, is one of the factors which determine the extent

of movement of its insertion, this shortening would tend to influence the range of action. In these cases, however, the disadvantageous position of the origin is compensated by the higher position of the insertion on the globe and the length of the muscle is not affected. The independent action of the normal inferior oblique is to elevate and abduct the cornea and cause the globe to revolve outward on its anteroposterior axis. The first and last

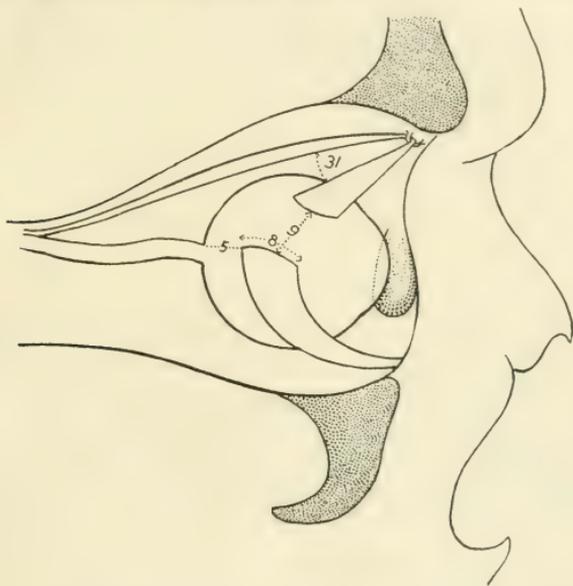


Fig. 3 Diagram to show an instance of abnormal origin of the inferior oblique muscle and its higher attachment onto the eyeball.

of these movements are not impaired by the higher position of the insertion; abduction is chiefly effected by the lateral rectus, though shared by the superior rectus, so that the effect on this movement is negligible.

The superior oblique muscles were examined in the same subjects, especially as in the first case the pulley for the muscle was situated further forward than usual, being almost on the orbital margin (though the position was normal in the other cases exam-

ined). Here the angle between the belly of the muscle and its reflected tendon was apparently less than usual, but accurate measurement was impossible, owing to the nobility of the globe in the advanced stage of the dissection. The angle was certainly much less than the normal 54° , and appeared about 31° . The insertion onto the globe in each of two cases was broader than usual (14 mm. and 11 mm., instead of an average width of 9.5 mm.), but the position as regards the vertical meridian was normal and not of the myopic type (i. e., parallel to and wholly on the lateral side of the meridian); as in the case of the inferior oblique, however, there is much variation in the 'normal' insertion.

From comparative anatomy it is seen that this condition resembles that found in certain fishes, where the oblique muscles arise from the orbital margin more anteriorly, in front of the globe.

RECTI MUSCLES

As regards abnormalities of the recti muscles, it is probable, to judge from the writer's individual experience in finding quite a number of gross anomalies in a series of dissections, that such are by no means as excessively rare as would appear from the number recorded in the literature; in the ordinary dissecting-room conditions do not favor their identification, and in life some may be unrecognizable through compensatory action of the other muscles. They can of course be explained by errors in development by cleavage from the common premuscular mesoblastic mass. In the following notes, mention will be made of such abnormalities as have been recorded in addition to those found by the writer.

The superior rectus has been found to give off a muscular slip 15 mm. long, which arose from the same origin from the annulus of Zinn and passed downward and forward across the lateral face of the optic nerve to join the inferior rectus about its midpoint; the nerve supply came from the inferior division of the third nerve (Aubaret, '09).

The medial rectus has been found absent in some cases of divergent strabismus (Ledouble, '97; Krause). Its posterior third

may be fused with the inferior rectus. A bifid sclerotic insertion by two tendons, 16 mm. in length, is recorded (Wieherkiewicz, '07).

The lateral rectus has likewise been found undeveloped in some cases of convergent strabismus (Ledouble, Krause), and a case of atrophy of the muscle has been noted on operating for strabismus in the living (Bourgeois). A fasciculus may pass from it to the inferior rectus, as is normal in certain ruminants, or to the lateral wall of the orbit (Moseley, '53). A lateral rectus with two extra fasciculi which passed forward to end on the inferior tarsal plate and lateral wall is recorded (Curnow, '73). In a specimen dissected by the writer there was a well-marked, fleshy bundle 7 mm. long and 2 mm. in diameter, passing from the lateral rectus across the posterior third of the orbit beneath the optic nerve to fuse with the belly of the medial rectus; no nerve could be traced to it.

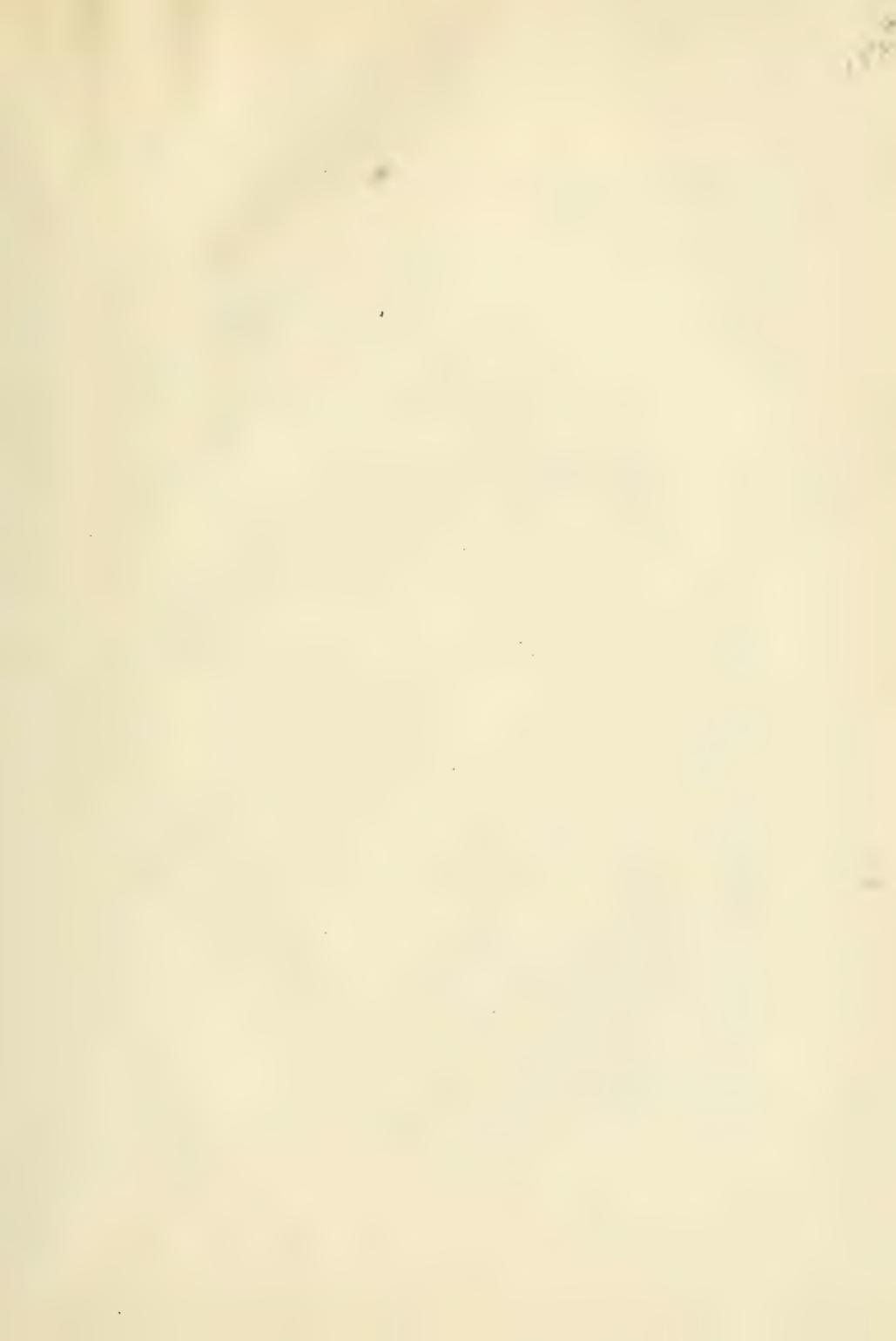
The inferior rectus has been found by the writer to give off a large muscular bundle which passed lateral to the optic nerve and joined the superior rectus; it was innervated by the lower division of the third nerve.

An abnormal muscle bundle (*musculus obliquus accessorius inferior*) has been found by Rex ('87) passing from the apex of the orbit to the inferior oblique, but also sending a slip to join the inferior rectus; it was found in both orbits, and was supplied by the third nerve.

As regards the vestiges of the *musculus retractor bulbi* which have been found in man, the reader should refer to an article by the writer ('11); to the literature therein cited may be added a paper by Hopkins ('16).

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Resumen por el autor, H. E. Radasch.

La determinación del tanto por ciento de substancia orgánica en el hueso.

El tanto por ciento de substancia orgánica en el hueso compacto es 32 a 33 por ciento, según los autores. De la inspección de la literatura general no se desprende el método empleado en la determinación de dicha substancia orgánica. Con el objeto de determinar el tanto por ciento real y hallar, a ser posible, el método empleado por los primeros observadores, el autor ha llevado a cabo varios experimentos de diversa naturaleza. Después de preparar cuidadosamente trozos del fémur, tibia y fibula, pesó una serie de trozos, que se calcinaron después, pesando el producto de esta operación. La pérdida de peso indica la cantidad de substancia orgánica incinerable que forma parte del hueso compacto.

Entre los veinte a sesenta años de edad, el tanto por ciento medio hallado es 40.75. En el gato adulto el tanto por ciento de peso en del hueso joven es 38.32, mientras que en el conejo (dos tercios del crecimiento total) el tanto por ciento medio es 38.90. Mediante otros métodos, la humedad y las substancias solubles en el alcohol y el éter fueron eliminadas, fijando la cantidad de contenido orgánico fijo. La cantidad media de humedad durante el periodo comprendido entre los veinte y sesenta años es 8.42%, y la relación 7 de la substancia orgánica fija y el hueso seco es solamente 34.92%. La cantidad media de substancia soluble en el éter es 9.27 por ciento; la relación media de la substancia orgánica fija y el hueso extractable es 31.34%. Parece sin embargo que el peso tipo debe ser el del hueso joven, y si se acepta esto, la cantidad media, de substancia orgánica contenida en él es 40.75 por ciento.

THE DETERMINATION OF THE PERCENTAGE OF THE ORGANIC CONTENT OF COMPACT BONE

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In considering the chemical composition of bone we are told that it consists of two main substances intimately commingled, viz., earthy and animal substances. The former comprises the following substances, according to Cunningham's Anatomy ('14):

	<i>per cent</i>
Calcium phosphate	53.23
Calcium carbonate	7.32
Calcium fluoride	1.41
Magnesium phosphate	1.32
Sodium chlorid	0.69
	<hr/>
	68.97
Organic material	31.03
	<hr/>
	100.00

The organic substances comprise fats and ossein.

According to Piersol, who quotes Berzelius, the inorganic material comprises 67.3 per cent, while the organic material consisting of gelatin and blood-vessels constitutes 33.3 per cent. Schaefer, Prudden (Ref. Handbook of Medical Sciences), Sobotta, all give the same as Piersol, apparently having accepted the same source as their guide. Gray's Anatomy ('18) gives the organic content as from 67 to 68 per cent and so the inorganic constitutes 33 to 32 per cent.

We are further told by Schaefer that the animal material, improperly called cartilage of bone, differs from cartilage physically and chemically. It is much more flexible and softer and upon boiling the bone yields mainly gelatin. He concludes that

the animal material closely resembles white fibrous and areolar tissues in that it consists mainly of collagen.

Normal bone is hard, rigid (to a certain extent), tenacious, and also elastic. The earthy materials contribute to its hardness and rigidity, while the organic material gives bone its tenacity and elasticity. With the last characters in mind, we can readily understand some of the results of fractures. Although we are not told so, we naturally conclude that the foregoing percentages and characteristics apply to compact bone and to the adult type. This being agreed, we naturally would consider that the bones of the young and adolescent would contain a greater percentage of organic material and, therefore, a lower percentage of inorganic substance. This chemical difference, therefore, makes a physical difference to the effect that the bones of the young should, theoretically, be more elastic and tend less to fracture under the same proportionate strain than that of the adult; that the writer believes the surgeon will admit. In the case of ultimate fracture, however, we might expect a different result than in the adult, and consequently we find the green-stick fracture pertains to youth entirely and does not occur in the adult. This is due to the higher percentage of organic substance in the bones of the young. This will be shown actually in the succeeding data.

We are told that by subjecting the bone to an open fire (calcining) the organic substance is burned out, leaving a white, brittle, chalk-like substance that preserves its original shape, but with the loss of about one-third of its weight. This porous cast is easily broken, so apparently the substance which gives tenacity has been removed. We might naturally infer from this that in old age there is a reduction of organic substance, for it is known that in old individuals fractures occur more easily than in those in the prime of life; also repair is less rapid and less satisfactory in old age. We would believe, then, in the elderly, that there is a reduction of organic substance that causes the bone to yield more quickly to strains. Yet Rusby (Ref. Handbook of the Medical Sciences) tells us that as age advances there is a diminution in the mineral constituent of bone and the

organic element is slightly increased. This should make the bone somewhat more tenacious and less prone to fracture—just the reverse of actual experience. He states that this increase is due to the replacement of bone by enlarged blood-vessels and marrow, so that there is, in reality, a reduction of fixed organic material. Yet in those bones examined in this work no reduction was noted, but rather an increase, so that theoretically the bone should not be weakened in old age as it naturally is. There is apparently a thinning or diminution of the actual bony layer of the long bone, and this accounts partly for the readiness of the fracture.

Hoppe-Seyler is quoted (Ref. Handbook of the Medical Sciences) as giving the following composition of dried bone without the removal of the marrow or blood. Water, 50 per cent; fat, 15.75 per cent; ossein, 12.40 per cent; bone earths, 21.85 per cent. Why the bone-marrow and blood should be considered and included in the determination of the organic composition of bone seems strange. These two substances are not a direct part of the bone and they should be got rid of entirely in the determination of the organic constituency. Again, these substances will give some ash and will contain some elements that are concerned in bone formation, so that the effect of calcination of fresh bone with its blood and marrow would be to add to the inorganic constituents the amount of ash of the organic parts. As a result, the percentage of inorganic would be somewhat higher than it should.

For the same reason there is no way in which the organic structure of cancellous bone can be determined, as the marrow contained cannot be got rid of in any way that would not tend to remove some of the soluble organic constituent of the bone. The percentage of organic material would be quite high in such a case.

It would seem that to get the real percentage of organic substance of compact bone, it would be necessary to remove as thoroughly as possible all traces of fat, marrow, and blood-vessels and external, or surface moisture. In that way the organic material remaining would practically belong to the bone and be a part thereof.

Primarily, the reason for the following determination was to find out, if possible, a definite relation, or variation, of the organic constituents of bone at the various ages. Just what methods of procedure were used by those who made the early determinations was not known, so some fresh bone was cleaned, weighed, calcined, and the contents determined. By this method the percentage of inherent, fixed, or component organic substance was so far above that given in the text-books that it caused surprise. There was no reason to believe that it could be an accidental variation, so that the writer immediately wondered how Berzelius, et al., made their determinations. If, according to Hoppe-Seyler, the marrow and blood-vessels were included with the bone, then we can see readily that there would be a variation, but this would make the organic content still higher than the author's determination.

In order to attempt to find out the method used by the older chemists, the determination of the organic content was carried on in a number of different ways.

So as to try to reach as near as possible the true percentage of organic substance in compact bone, five different sets of determinations were carried out. Four of these were applied to every sample of green bone and the fifth applied to the bones used for study by the students. The determinations were made on the bones of adults, varying the ages as much as possible, and stillbirths and even fetuses. In addition, in order to have a comparative anatomy relationship, determinations were also made in rabbits and cats.

The same method of preparation was used in all, except the bones used by the students for study. Certain bones only were used in all ages and in all of the animals, viz., femur, tibia, and fibula. In order to get as fair a sample of compact bone, i.e., where it would be most compact, the middle (from end to end) of each was chosen, and a section cut out and handled in a certain routine manner in all instances. The material was chosen chiefly from postmortem subjects, and not from the remains of the subjects from the dissecting-room for several reasons: 1) To avoid involving the chemicals of the embalming fluid. 2) In

order to determine the normal inherent moisture of the bone as just removed from the body. Determinations were also made in bodies that had been refrigerated for some time, and a difference was noted in the results here also.

After removal of a section of the femur, tibia, and fibula from the same sources, the flesh was allowed to remain on the others until one had been prepared for all four methods, as follows: First, the flesh of one piece of bone is all carefully cleaned off and the periosteum completely stripped, say off the femur. This caused no trouble except along the *linea aspera* of the femur and at all of the borders of the tibia and the fibula. Here the membrane adheres most tenaciously, as many processes extend into the bones at these lines and serve to anchor the membrane firmly in position. Here care must be exercised to get out all traces of Sharpey's fibers, else they might naturally add their mite to the organic constituent of compact bone, and erroneously so.

The next step is to strip out all of the marrow and then cut out all of the cancellous spicules along the inner surface of the bones. In cutting these away considerable fatty matter is thus exposed and removed, thereby getting rid of another error-producing element. When as much of this cancellous bone as possible has been removed, one can feel reasonably sure that the remainder is a real sample of compact bone. Then the bone is carefully wiped externally and internally and a section (ring) about 1 cm. in height is cut; this is then cut into quarters. Each quarter is then carefully wiped again, especially the narrow-cavity side, in order to remove all superficial moisture and fat; it is then weighed and this is the green weight. This is done with all four pieces, so that this green weight serves as an excellent check in the three other determinations.

Next the tibia is treated in the same way and then the fibula. The reason why all are not cleaned at once and then cut and weighed is because, if cleaned and left exposed to the room air, some of the moisture would escape and cause a variation in the determination. For that reason each specimen is treated in this routine manner. This may seem far-fetched, but it is of

the greatest importance, especially in the handling of fetal bones and bones of the child at birth. Here the parts must be cleaned, wiped, and weighed as rapidly as possible, or there is a marked variation, as these young bones have a greater percentage of inherent moisture. If the weighing process is slow, this variation will be noticed right on the balance pans.

By this method pieces of adult bone weighing from 1.1 grams up to over 3 grams were prepared. In fetal bones and those of the cat and rabbit, however, the weight of green bone was about 0.5 gram, as they are very bulky for their weight.

To procure and prepare four pieces of femur, tibia, and fibula and get the green weight of each requires about two hours of tedious and patient labor, but this is not the end.

The importance of the removal of all of the cancellous tissue and contained marrow will be shown in determinations A, B, C, and D. It will also show why cancellous bone proper should not be used, as the true organic constituency of osseous tissue cannot be correctly determined therefrom. This is due to the inability to get at and to remove the marrow from the cancelli.

Each of the four pieces of sample was treated as follows:

1. Green. The first piece of each bone was immediately calcined until the weight was constant and then the percentage of organic material determined, as will be shown later.

2. Oven-dried. The second piece of each bone after preliminary weighing was placed in an oven at 56°C. for twenty-four to forty-eight hours, allowed to cool, and then weighed. The loss in weight indicated the amount of moisture and the volatile organic substance present. It was then calcined until the weight was constant. By this method the percentage of moisture and volatile matter was found and also the amount of what might be termed real, or fixed organic material was obtained. In addition the green (original) weight permitted a determination of the organic material in green bone before the drying process was undertaken, giving a green-check determination.

3. Alcoholic-extracted and oven-dried. The third piece of each bone (after preliminary weighing) was placed in a 95 per cent

alcohol for twenty-four to forty-eight hours and then transferred to an oven at 56°C. for twenty-four to forty-eight hours, and then weighed when cooled. The weight lost indicated the moisture and volatile material and alcohol-soluble material. After calcining then the percentage of organic material in green bone, the percentage of moisture, volatile material and alcohol substance was next determined, and lastly the amount of remaining (fixed) organic substance.

4. Ether-extracted and oven-dried. In this determination a fresh piece from each bone (after preliminary weighing) was placed in ether from twenty-four to forty-eight hours, then oven-dried at 56°C. for twenty-four to forty-eight hours, and then weighed when cool. After calcination the percentage of organic material in green bone, the percentage of moisture, ether-soluble and volatile materials was next computed and then the amount of (fixed) organic substance remaining was computed.

The calcination was carried out by the use of two Bunsen burners. The porcelain crucible containing the green or extracted bone was placed on a triangle at an angle of about 45° and just high enough so that the light blue cone was close to the crucible. The crucible and contents were warmed gently and then the burner put in a position with some air cut off so that for the first half hour the heat would not be so intense, but sufficient to volatilize and drive off most of the carbon and volatile substances. These would ignite and burn at the mouth of the crucible. Following this, the air was turned on full to get the greatest heat and another Bunsen with all of the air turned on was held so that the blue cone was directed upon the piece of bone at the mouth of the crucible. Between these two flames the calcining was completed; the bone being turned from time to time. In this way the bone is rendered incandescent and all carbon is burned out. The time varies for different thicknesses from five minutes to half an hour. This is repeated until the weight is constant. The heat should be carefully applied at first.

In order to comprehend the following tables the method of determining these various percentages will be first given by an example:

Body 89—1920	Negro	Frozen	About 35 years of age	
Bone No.	Ether—Oven-dried			
Femur 90	31.6305	31.6305	4.0670 a	4.0670 a
	27.5635	27.9450	3.6845 b	2.4029 d
	4.0670 a	3.6845 b	0.3825 c	1.6641 f
	31.6305	3.6845 b	f/a = 40.90 per cent organic material in green bone.	
	29.2276	2.4029 d	c/a = 9.55 per cent moisture, ether-soluble substance, etc.	
	2.4029 d	1.2816 e	e/b = 34.78 per cent fixed organic material in the extracted bone.	
			e/a = 31.51 per cent of fixed organic material in reference to green bone.	

In all of the weighings a constant weight (50 grams) was used on the left pan and the right one contained the watch-glass, bone, and weights required to balance the 50 grams.

The various letters indicate the following:

- a = The weight of the green bone.
- b = The weight of the bone after (1) oven-drying or (2) alcohol-extracting and oven-drying or (3) ether-extracting and oven-drying.
- c = The weight of the moisture and volatile material alone or (1) + the alcohol-soluble or ether-soluble material.
- d = The weight of the calcined bone.
- e = The weight of the organic material after (1) oven-drying, or (2) after extracting and oven-drying.
- f = Organic material in green bone.
- f/a = The percentage of organic material and water, volatile and extracted material in green bone.
- c/a = The percentage of water, volatile and extractable material in green bone.
- e/b = The percentage of fixed organic material in the dried or extracted and dried bone.
- e/a = The percentage of fixed organic in the green bone.

These last two percentages exclude the water and volatile material, and while one percentage is in relation to the weight of the extracted bone, the other percentage is in relation to the weight of the green bone.

The results obtained will be tabulated with reference to age and method employed. *Fresh* indicates simple postmortem, not embalmed and not preserved in any way; *frozen* indicates storage

in the cadaver refrigerator and unembalmed; *dissection* refers to material taken from cadaver after the dissection was completed.

Green bone

Four and one-half months (fresh)

NUMBER	BONE	f/a	REMARKS
7	Fe	63.35	Fresh
8	Tib	66.05	

At birth (fresh)

1	Fe	49.61	Extremity of femur consisted mostly of cancellous bone and contained marrow and blood
2	Tib	50.44	
55	Fe	50.79	
56	Tib	53.07	
A		65.95	

20 to 60 years

84	Fem	41.15	Negro
85	Tib	41.95	47 years
84a	Fib	39.85	Frozen
112	Fem	40.61	Dissection
113	Tib	41.32	
113a	Fib	40.98	

61 to 90 years

11	Fe	40.88	71 years
15	Tib	42.46	Frozen
19	Fem	41.57	Very greasy
23	Feb	44.18	Fresh
24	Tib	44.68	Bones thin, 87 years
31	Fem	43.75	Frozen
35	Tib	45.25	
39	Fib	41.56	
43	Fe	42.28	Fresh. 76 years
44	Tib	41.40	
45	Fib	41.00	
63	Fe	48.62	80 years
64	Tib	45.77	Fresh. Bones thin and buckled in calcining
66	Fib	41.48	

Green Bone—Continued

61 to 90 years

NUMBER	BONE	f/a	REMARKS
76	Fe	39.81	
77	Tib	41.68	Frozen. 87 years
77a	Fib	40.71	
92	Fe	39.44	65 years
93	Tib	41.78	Negro. Frozen
100	Fe	43.39	
101	Tib	39.86	Fresh. 89 years
101a	Fib	40.80	

Oven-dried bone

Four and one-half months

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
9	Fe	61.23	27.41	46.59	33.82	
10	Tib	65.35	35.04	46.67	30.31	Fresh

Eight and one-half months and full term (at birth)

108	Fe	52.20	21.50	39.34	30.88	8½ months
109	Tib	53.01	22.04	39.70	30.98	Fresh
3	Fe	49.22	16.03	39.50	33.19	At birth
4	Tib	50.88	17.15	40.73	33.74	Fresh
57	Fe	52.20	19.34	40.72	33.00	At birth
58	Fib	54.95	25.52	39.55	29.46	Fresh
B		66.61	41.59	44.04	25.72	Extremity femur, mostly cancellous bone

20 to 60 years

86	Fe	40.57	8.36	35.15	32.20	
87	Tib	40.95	9.29	35.39	31.66	
87a	Fib	38.99	8.27	33.49	30.72	
114	Fe	40.28	9.22	34.21	31.06	
115	Tib	40.77	9.59	34.13	30.79	
115a	Fib	40.43	8.34	35.03	32.09	
122	Fe	42.31	7.71	37.01	33.80	
123	Tib	40.65	6.32	36.25	34.24	Dissection
123a	Fib	41.04	7.85	36.02	33.49	

Oven-dried bone—Continued

61 to 90 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
12	Fe	40.70	8.42	35.36	32.44	
16	Tib	40.48	lost	lost	lost	
16a	Fib	43.36	8.70	37.96	34.66	
25	Fe	48.63	9.86	43.51	39.65	
26	Tib	46.51	7.61	42.10	38.89	
32	Fe	42.28	3.48	40.11	38.66	
36	Tib	42.38	8.04	37.34	34.34	
40	Fib	40.00	6.85	35.58	33.14	
46	Fe	41.19	9.23	35.22	31.97	
47	Tib	42.41	11.55	34.61	30.62	
48	Fib	41.10	9.78	34.71	31.32	
67	Fe	51.50	15.16	42.52	36.10	Moisture very high, bones shrunk as they calcined
68	Tib	45.45	11.00	36.76	32.72	
69	Fib	41.85	27.76	19.49	14.82	
78	Fe	41.62	12.94	37.74	35.70	
79	Tib	39.76	7.81	34.67	31.96	
79a	Fib	39.20	5.79	35.29	33.39	
94	Fe	40.01	7.01	35.53	33.05	
95	Tib	39.22	6.80	34.77	32.41	
95a	Fib	40.09	7.82	35.15	32.48	
102	Fe	40.86	6.40	36.84	34.10	
103	Tib	41.42	6.59	37.29	34.83	
103a	Fib	41.44	7.94	36.39	33.38	

Alcohol-extracted and oven-dried bone

At birth

5	Fe	53.67	23.63	38.70	29.56	
6	Tib	53.81	30.08	39.46	27.58	
59	Fe	50.36	23.37	38.53	30.32	Extractives high. Can- cellous bone in one end of sample
60	Tib	50.60	19.88	38.34	30.74	
C		68.81	44.92	43.51	23.89	Extremity of tibia. Lots of extractives and mois- ture

Alcohol-extracted and oven-dried bone—Continued
20 to 60 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
88	Fe	39.66	6.28	35.22	33.00	
89	Tib	39.45	6.87	35.01	32.61	
89a	Fib	38.80	8.27	33.49	30.72	
116	Fe	40.62	8.99	35.09	32.19	
117	Tib	41.44	9.60	35.73	32.77	
117a	Fib	40.09	11.17	32.69	32.42	
124	Fe	42.85	8.35	37.75	34.61	
125	Tib	41.07	7.24	35.94	33.28	Dissection
125a	Fib	41.78	9.37	35.76	32.33	

61 to 90 years

13	Fe	40.01	7.34	35.21	32.66	
17	Tib	43.46	9.52	37.41	33.86	
21	Fe	41.41	5.53	37.98	35.58	
27	Fe	46.55	10.54	40.20	36.04	
28	Tib	47.36	10.55	41.40	36.21	
33	Fe	40.89	7.11	36.37	33.79	
37	Tib	45.01	9.49	39.25	35.53	
41	Fib	41.24	8.46	36.31	32.50	
49	Fe	41.27	9.88	34.83	31.38	
50	Tib	42.09	11.96	24.22	30.12	
51	Fib	41.71	10.73	34.69	30.89	
70	Fe	47.42	8.37	42.45	38.90	
71	Tib	44.77	15.41	34.71	23.78	Eccentric results
72	Fib	43.98	11.74	36.37	32.10	
80	Fe	41.02	6.90	36.60	34.13	
81	Tib	41.28	8.47	35.84	31.77	
96	Fe	40.20	7.47	35.48	32.85	
97	Tib	40.36	7.31	35.94	33.31	
97a	Fib	40.17	6.09	36.28	33.92	
104	Fe	41.04	8.08	35.86	32.19	
105	Fib	41.11	8.34	35.75	32.77	
105a	Tib	40.42	8.00	35.24	32.42	

Ether-extracted and oven-dried bone

Eight and one-half months and full term (at birth)

NUMBER	BONE	f/a	c/a	c/b	c/a	REMARKS
110	Fe	51.35	20.04	38.95	31.14	8½ months. Fresh
111	Tib	52.44	21.53	39.38	30.73	
61	Fe	53.56	23.85	31.35	29.71	Extremity of tibia mostly cancellous
62	Hum.	54.48	23.35	40.43	30.91	
D		66.57	41.54	41.68	24.96	

20 to 60 years

90	Fe	40.66	9.55	34.78	31.51	
91	Tib	41.63	10.40	34.88	31.25	
91a	Fib	39.46	8.01	34.19	31.45	
118	Fe	41.46	9.43	33.81	30.62	
119	Tib	39.56	9.01	33.94	30.87	
119a	Fib	39.49	8.32	33.36	31.17	
126	Fe	42.59	11.58	35.08	31.01	
127	Tib	41.32	8.18	36.66	33.23	

61 to 90 years

14a	Fe	41.84	8.14	36.69	33.70	Frozen
18a	Tib	41.98	7.67	36.85	33.86	
22	Fib	40.76	6.35	36.69	34.40	71 years
29	Fe	42.11	9.03	35.23	32.47	87 years Extractives high
30	Tib	42.48	11.29	35.17	31.18	
34	Fe	46.62	8.77	34.29	27.86	
38	Tib	39.13	8.80	33.43	30.48	
42	Fib	44.70	15.59	34.49	29.09	
52	Fe	41.42	11.01	34.19	30.41	
53	Tib	41.25	11.64	33.52	29.61	
54	Fib	41.29	10.80	34.18	30.49	
73	Fe	42.85	15.23	32.58	27.62	
74	Tib	47.89	13.57	39.70	33.56	
75	Fib	43.00	15.64	32.54	27.41	

Ether-extracted and oven-dried bone—Continued
61 to 90 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
82	Fe	43.72	13.37	35.05	30.35	
83	Tib	43.16	13.26	34.47	39.89	
83a	Fib	41.45	12.43	33.15	29.04	
98	Fe	40.60	8.17	35.37	32.50	
99	Tib	42.31	10.24	35.73	31.71	
99a	Fib	40.28	9.04	34.36	31.25	
106	Fe	41.21	10.26	34.49	30.05	
107	Tib	41.11	10.40	34.82	30.71	
107a	Fib	40.61	9.45	34.01	31.26	

Cleaned bones of study sets

NUMBER	BONE	f/a	REMARKS
160	Fe	36.73	Quite clean, dry, apparently free from grease, the tibia had a little marrow and was greasy around the marrow cavity
161	Tib	36.66	
162	Fib	37.80	
163	Fe	36.15	Fairly clean
164	Tib	35.72	Clean
165	Fib	34.41	Very clean and dry, like ivory
166	Fe	39.70	Surface greasy to the touch
167	Tib	41.54	Very greasy to the touch. Only partly degraded in the boiling
168	Fib	42.08	

Green bones

Rabbit (two-third grown)

501	Fe	35.87	
505	Tib	41.74	
509	Fe	37.00	
513	Fe	37.66	
517	Tib	41.43	

Cat (adult)

530	Fe	39.43	
531	Tib	39.69	

Oven-dried bones
Rabbit (two-thirds grown)

NUMBER	BONE	f/a	c/a	c/b	e/a	REMARKS
502	Fe	35.11	7.85	29.59	27.76	
506	Tib	35.08	5.67	31.15	29.41	
510	Fib	38.90	10.16	31.98	28.74	
514	Fe	40.64	12.81	31.91	27.82	
518	Tib	42.07	12.99	33.37	29.04	

Cat (adult)

532	Fe	39.10	9.75	32.37	29.31	
533	Tib	39.01	8.75	33.13	30.24	

Alcohol-extracted and oven-dried bones

Rabbit (two-thirds grown)

503	Fe	38.78	11.79	30.64	26.98	
507	Tib	38.54	10.58	31.28	27.77	
511	Fe	34.97	9.82	27.88	25.14	
515	Fe	38.88	11.54	30.57	27.04	
519	Tib	40.35	11.33	32.61	28.86	

Cat (adult)

534	Fe	39.28	10.40	32.17	28.81	
535	Tib	39.49	9.08	33.70	30.75	
535x	Tib	43.88	13.03	35.65	31.09	Extremity containing considerable cancellous bone and marrow

Ether-extracted and oven-dried bones

Rabbit (two-thirds grown)

508	Tib	41.28	12.27	33.06	28.95	
512	Tib	38.25	9.38	31.88	28.87	
516	Fe	40.24	12.18	33.54	28.08	
520	Tib	41.80	12.56	33.55	29.38	

Cat (adult)

536	Fe	39.04	9.90	32.35	29.14	
537	Tib	38.32	9.60	32.14	29.22	
537x	Tib	42.09	11.29	34.01	30.79	Extremity of shaft with cancellous bone and marrow

DISCUSSION

Green bone

By making a green weight of all pieces of bone used for these determinations, a normal green-weight percentage of the organic material is obtained in all of the different methods of after-treatment. This not only gives a greater number of results (or a better average) for this determination, but also acts as a check for the other methods. In the opinion of the writer, this green determination is the one that should be used in referring to the organic and inorganic constituents of compact bone.

Fetal bone (four and one-half months)

Upon examining the results of the determinations upon green bone, some very interesting facts are brought out. In the fetus we find that the percentage of organic substance is very high (63.99 per cent), the bone practically consists of two-thirds organic material in the fetus at four and one-half months. This leaves one-third inorganic substance. This is natural, as the bone, before its ossification, is purely organic material. In making this examination great care had to be exercised in preparing the specimen and the weighing had to be rapidly made, as the drying upon the scale pan could be noted. Again, the piece of bone free from the cancellous tissue and marrow necessarily could not be large, as the bones at that age are small.

Bones at term (eight and one-half to ten months)

At eight and one-half months and birth we note that the average is 52.15 of organic material. This is an increase in the percentage of inorganic material with a variation from 49 per cent, or practically half organic and half inorganic substances. This variation, of course, is reasonable, as the diet of the mother has a great influence upon the hardness of the bones of the child. If the mother's diet be rich in bone-forming elements, no doubt the percentage of inorganic material would be raised beyond the above figures.

In this table is the determination of bone A. Here the percentage is 65.95. Why is it so high, practically that of the fetus of the fourth to the fifth month? The note in connection with the determination states that this specimen was the epiphyseal extremity of the diaphysis (in contradistinction to the central part usually used) and that it consisted mainly of cancellous bone with its contained marrow. The percentage is practically 15 per cent above the average—a variation too great to be overlooked.

It will be remembered that at the beginning of this paper it was stated that all cancellous bone was removed before the bone was weighed, because cancellous bone contains marrow which could not be removed independently of the bone, and its presence would increase the percentage of organic material if left in the specimen. Marrow is not an organic constituent of bone and must, therefore, be removed. If it is not got rid of, then an inflated per cent of organic content is the result. The determination in the fetal bone (A, table 1) is an excellent example and proof that if a specimen contains cancellous bone, it should be entirely discarded or the cancellous bone should be completely removed. Hence the care exercised in the preparation of all specimens before weighing. This same proportionate increase will be seen in the companion determination B, C, and D.

Green bone (20 years to 61 years)

In this group the average per cent is 40.75 of organic substance. These determinations are quite close. The lowest is 38.80 per cent and the highest is 42.85, while most of the determinations are between 40 per cent and 41 per cent. In reference to these variations it might be noted that the tibia giving 38.80 per cent was from an individual 47 years of age and all other green determinations ran along this same rate, all under 40 per cent, showing that the whole series was consistent and not an individual variation due to an accidental loss. In reference to the 42.85 per cent, it might be noted that the age was 60 (border line), and again all but one were above 42 per cent, again showing a consist-

ent figure and not an abnormal variation. Now (this might be due to an unusually great amount of fat in the marrow and to unusually large haversian canals in the neighborhood of the marrow cavity, containing an unusually large amount of fatty marrow; or again, it might indicate a border-line case in which there is a slight increase of organic material in old age and in this particular individual this increase came on a little earlier than usual (an early senescence). In the next series the percentage of one case of 65 years varies between 39 and 40, and, therefore, resembles those of the younger group, indicating a comparatively youthful state.

Green bone (61 to 90 years)

In this group the ages are 65, 71, 76, 80, 87, and 89 years, giving a fairly varied series. Here we find the lowest to be 39.13 in one of 87 years. Determinations were made on the fresh subject and after the remains had been in the cold storage for quite a while; while in the fresh state, the green-bone percentages were consistently high, after freezing the percentages were consistently lower and more like 20 to 61 years of age. A variation in the moisture was also noted under these different conditions.

The highest was 51.50 and 48.03 per cent in two different individuals aged 80 and 87 years, respectively. These are isolated instances which might fairly be thrown out, but still they were retained and included in the series for the reason that this moisture and extractive content were relatively high in each case, seeming to indicate that these figures were not accidental, but in keeping with the other percentages in the experiments. In connection with most of the specimens of the individual aged 83 years, it might be mentioned that upon calcination the bones shrank, curled, or buckled and reacted like the fetal and animal bones where the organic content is relatively high or the bones are relatively thin.

Oven-dried bone

In order to try to obtain as complete a series of determination as possible, the moisture and volatile organic substances were first removed and then fixed the organic content determined. After the green weight was determined, then all of the desired specimens were subjected to the same heat (drying oven at 56°C.) to remove the moisture and ordinary volatile material (if any such were present). Then by weighing and subtracting the weight from the original green weight, the weight of the moisture and ultimately its percentage were readily determined. Then the amount or weight of the fixed organic material can be obtained. From the weight two results may be deduced: *a*) the percentage of organic substance to green bone and, *b*) the percentage of organic substance to the dried bone (green bone less its moisture). These three percentages will be taken in order.

Moisture and volatile organic material (c/a)

Four and one-half months. Here we find the percentage of moisture to average 32.43 per cent. This seems high, but we must remember that the bones are still two-thirds organic material, and organic material, in general, contains a large percentage of free water. We must also remember that the mesenchymal tissues at this stage are largely undifferentiated and so mainly embryonic in character and of a higher water content than at birth and later. We should, therefore, naturally expect a higher moisture percentage.

Eight and one-half months and full term. Here the average is 20.26 per cent with a variation from 16.03 to 25.52. By consulting the table it will be noted that the femur has a lower moisture content than the tibia, probably because the former is older from the developmental standpoint. It will also be noticed that in the eight and one-half months' fetus, the percentages are between those of the two full-term fetuses. This evidently again indicates a difference in the maternal diet. The specimen B represents the epiphyseal extremity of the diaphysis and it consisted mainly of cancellous bone and its contained marrow.

In the moisture content was 41.59 per cent due to the great quantity of blood that could not be removed. This shows why cancellous bone should not be used.

20 to 60 years. In this group while the average is 8.44 per cent, the highest is 9.59 per cent and the lowest 6.32 per cent. The highest amounts were usually noticed in the fresh (unfrozen or unembalmed bodies) and the lowest in those that had been frozen or embalmed. While there seems to be an extraction of moisture from the bones during storage in the refrigerator and an abstraction of moisture from the bones by the embalming fluid in many of the determinations, it is not constant, as will be pointed out later.

61 to 90 years. In this group there is a remarkable variation in moisture content. The average is 8.89 per cent, while the highest is 27.76 per cent and the lowest 3.48 per cent. The former occurred in the tibia of the 80-year-old individual, and this specimen curled and buckled when calcined. The lowest percentage (3.48) occurred in the 80-year-old individual after freezing, and yet other determinations of the same gave as high as 12.94 per cent. This body showed erratic results, so a number of determinations were made by various methods and all were included in the averages.

Although the average of moisture in the group 61 to 90 years is only 0.45 per cent higher than in the 20 to 60 years' group, still that little represents what the proportionate difference should be in comparing their green weights of 40.05 per cent and 42.24 per cent, respectively.

Organic material less water and volatile material in oven-dried bone

Having removed the water and volatile material, the weight of the calcined bone may now be compared with the original green weight and also with the oven-dried weight.

Relations of the organic (less moisture) to oven-dried bone (e/b)

Four and one-half months. If now we determine the percentage of organic substance after removing the water in the oven-dried bone, the average is 46.63 per cent.

Eight and one-half months to full term. In this group the average is 39.92 per cent with a variation from 39.34 per cent to 40.73 per cent—a fairly uniform series.

20 to 60 years. In this group the average is 34.52 per cent with a variation from 33.49 to 37.01 per cent. The former occurred in an individual of 35 years and the latter is one of 60 years.

61 to 90 years. In this group the average is 36.22 per cent with a variation from 19.49 per cent to 43.50 per cent. The cause of the extreme variation could not be determined and, strange to say, the average of these two extremes is nearly the general average.

Relation of organic material (less moisture) to the green weight (e/a)

Four and one-half months. In this group the average is 32.06 per cent. By adding this to the percentage of moisture, the result is 63.49 per cent of combined moisture and organic substance proper. The amount given is 63.29 per cent as per the table. All these percentages were obtained by dividing one number into the other and not by mere subtraction, hence the sum of e/a and c/a are not always equal to f/a as they would be by mere subtraction.

At eight and one-half months and full term. The average here is 31.88 per cent with a variation from 29.46 per cent to 33.74 per cent.

20 to 60 years. In this group the average is 32.23 per cent with a variation from 30.72 per cent to 34.24 per cent. The highest were those in the individual of 60 years, the lowest in those of 35 years.

Alcohol extracted and oven-dried bone

This series consists of the results of treating green bone with alcohol for twenty-four to forty-eight hours and then drying for twenty-four to forty-eight hours in an oven at 56°C. and weighing to determine the amount of moisture and alcohol-extractable substances and later the 'fixed' organic substance (in relation to the green weight and the extracted weight).

Moisture and alcohol soluble extract (c/a)

At full term. The amount of moisture and alcohol-soluble material averages 24.24 per cent in the child at birth. The lowest is 19.88 per cent and the highest 30.88 per cent. This average represents 3.98 per cent more than the mere moisture and volatile material in oven-dried bones. This does not indicate a high percentage of soluble material, indicating some little fat or oil in the compact bone proper.

20 to 60 years. In this group the average is 8.46 per cent of moisture and alcohol-soluble material. The extremes are 6.28 per cent and 11.17 per cent. The average highest is in those bones of the individual of 35 years of age, while in the subject of 60 years of age they are intermediate. The average is almost identical with the average per cent of plain moisture and volatile substance (8.44), arguing that in individual cases there may be a difference, but in the main (average) that the alcohol-soluble material is very small in quantity.

61 to 90 years. In this group the average is 9.01 per cent with variations from 5.53 per cent to 14.51 per cent. This is higher than the preceding by a half per cent and is only 0.12 per cent above the plain moisture of the oven-dried group. Evidently in the adult the compact bone contains exceedingly little alcohol-soluble substance.

Fixed organic material in alcohol-extracted and oven-dried bone
Relation of the organic substance to the extracted and
dried bone (e/b)

At full term. In this group the average is 38.76 per cent. This is only 1.16 per cent less than the same determination in plain oven-dried bone.

The average here is 29.55 per cent. This is the real organic substance in compact bone of this age. This is 1.51 per cent lower than that of the plain oven-dried bone, indicating some alcohol soluble material in the bone at birth.

20 to 60 years. In this group the average is 32.66 per cent with variations from 30.70 to 34.61 per cent. This is 0.69 per cent

lower than that of oven-dried bone, meaning that there is very little alcohol-soluble substance in compact bone.

61 to 90 years. In this group the average is 32.67 per cent with variations from 23.78 to 38.90 per cent. Compared with the corresponding determination in oven-dried bone, it is 0.59 lower than that.

Ether-extracted and air-dried bone moisture and ether-soluble substance (c/a)

At term. The average of moisture and ether-soluble substance in compact bone at birth averages 22.19 per cent with variation of 20.04 per cent to 23.85 per cent. This is 2 per cent lower than alcohol-extracted bone and about 2 per cent higher than mere oven-dried term bones.

20 to 60 years. In this group the average is 9.27 per cent with variations from 8.18 to 11.58 per cent. This average is 1.19 per cent higher than alcohol-extracted bone and 1.21 higher than oven-dried bone.

61 to 90 years. In this group the average is 10.87 per cent with variations from 8.17 to 15.60 per cent. This average represents 1.86 per cent and more than that of alcohol extracted bone and 1.98 per cent more than oven-dried bone.

Relation of fixed organic material in ether-extracted bone to ether-extracted and oven-dried bone (e/b)

At term. This average is 37.53 per cent as compared with 38.76 in the alcohol-extracted bone and 39.92 per cent in oven-dried bones.

20 to 60 years. The average in this group is 34.46 per cent as compared with 35.07 per cent in alcohol-extracted and 36.52 per cent in oven-dried bones.

61 to 90 years. Here the average is 34.83 per cent as compared with 37 per cent in alcohol-extracted, and 46.22 per cent in plain oven-dried bones.

Relation of fixed organic material in ether-extracted bone to the green weight (e/a)

At term. The average is 30.60 per cent as compared with the average of 29.55 per cent of alcohol-extracted bone and 31.88 per cent in oven-dried bones.

20 to 60 years. The average is 31.34 per cent as compared to 32.66 per cent in alcohol-extracted bones and 32.23 per cent in oven-dried bones.

61 to 90 years. The average is 30.82 per cent in this group. This is to be compared with 32.67 per cent in alcohol-extracted bones and 33.26 per cent in oven-dried bones.

Cleaned bones

In order to make the study as complete and varied as possible, it was decided to take some of the bones of the study collection sets and determine the organic content of so-called cleaned bones. For this purpose three different sets were chosen, a piece of femur, tibia, and fibula in each set to conform to the preceding tests. One set was as clean and dry as possible (A), a set that showed grease and was greasy to the touch, yet cleaned as the others had been (C), and, thirdly, an intermediate set that looked fairly clean and yet gave indication of some grease (B).

B

NUMBER	BONE	f/a	REMARKS
160	Fe	36.73	These seemed quite clean and were apparently free from grease. The tibia contained a little dried marrow and was greasy around the marrow cavity
161	Tib	36.66	
162	Fib	37.80	

A

163	Fe	36.15	The femur was fairly clean
164	Tib	35.72	The tibia was quite clean looking
165	Fib	34.41	The fibula was very clean and dry and looked like ivory

C

166	Fe	39.70	The surface of the femur was greasy to the touch.
167	Tib	41.54	The tibia and fibula were very greasy to the touch and only partly degreased in the cleaning
168	Fib	42.08	

In the test upon the fresh uncleaned bones the corresponding results are as follows:

(e/b) in oven-dried bones:	
20 to 60 years	<i>per cent</i> 34.52
61 to 90 years	36.22
(e/b) in alcohol extracted and oven-dried bones:	
20 to 60 years	35.07
61 to 90 years	37.00
(e/b) in ether-extracted and oven-dried bones:	
20 to 60 years	34.46
61 to 90 years	34.83

In examining these results, interesting facts are noted. In group A, although the bones seemed clean, dry, and free from grease, the results varied. The tibia was the best looking and showed that all of the soluble and volatile substance had been removed and only the fixed organic material had been left. The percentage of fixed organic material in this bone was 34.41 per cent. This is practically identical with the percentage of fixed organic material in the ether-extracted bone and seems to indicate the complete extraction of all but the fixed organic substance. As we look through the other percentages we see the effect of the presence of unnoticeable and distinctly noticeable quantities of grease that remain through the incomplete cleaning of bones. Naturally, any such retained grease will have weight and will necessarily be included in percentage of fixed organic substance, though it should not be so included.

Another interesting fact is that in cleaning bones by boiling or macerating in water alone, all bones of the same body do not degrease or clean equally. Again some bones on repeated treatment seem to retain the grease to a considerable extent.

From this series it will readily be granted that the ordinarily cleaned bones are not satisfactory as a standard for the determination of organic substance in compact bone for bones that seem dry, clean, and free from grease may contain 1 or 2 per cent, and so change the final determination.

Other mammals

As some rabbits and cats were available at the time these determinations were made, it was decided to carry out the same series of tests upon the corresponding bones of these animals. The bones were prepared and treated in the same way and the tests were identical in all respects. The bones of these animals, however, are more difficult to handle. They are thin and bulky so that the amount of bone by weight was not great, varying from 0.1 gram to 0.5 gram. This did not allow a very great leeway for variations. Another prominent feature was that the bones at the first heating curled and split, and if the heat was not cautiously applied, small pieces might readily fly off and be lost. Also at the first heating the small areas of the bone that touched the crucible seemed to stick, but this did not seem to make any appreciable variation in the result.

The bones clean readily, as the periosteum strips with ease and the marrow comes out in toto as a plug would. There is no cancellous bone around the narrow cavity to be whittled away as in the human bone, so that the preparation of these bones is not so tedious a process as in the case of the human bones.

The weighing, however, must be rapidly carried out, as in the case of fetal bones, for these bones seem to contain a little higher percentage of moisture (as fetal bones) than the adult human bone. Perhaps this is only apparent and their thinness gives a greater proportionate surface for evaporation and so influences the result. There is no difficulty in getting the real green weight, however.

Rabbit. The rabbits used were all about two-thirds grown and represent the human at about the age of puberty. They were all in good health and were well developed. They had been utilized for other experimental work, but this had no influence upon the tests in hand.

Green bone. In this group the average of all of the bones tested was 38.90 per cent of organic substance, somewhat lower than we would expect to find it at the corresponding age of the human being.

The other determinations will be considered as follows:

Moisture and volatile and extractable substances

In oven-dried bone the average was 9.89 per cent.

In alcohol-extracted and oven-dried bone the average was 11 per cent.

In ether-extracted and oven-dried bone the average was 11.60 per cent.

Relations of the fixed organic substances to the extracted and dried bone (e/b)

In oven-dried bone the average was 31.60 per cent.

In alcohol-extracted and oven-dried bone the average was 30.58 per cent.

In ether-extracted and oven-dried bone the average was 33.01 per cent.

Relation of the fixed organic substance to the green bone (e/b)

In oven-dried bone the average was 28.56 per cent.

In alcohol-extracted and oven-dried bone the average was 27.16 per cent.

In ether-extracted and oven-dried bone the average was 28.82 per cent.

Cat. In the course of the laboratory work several cats were used for tissue purposes and so at the time the corresponding bones were prepared, as in the human, for these determinations. They were of adult age, well developed and apparently healthy.

Green bone. The average of all the bones tested was 38.32 per cent—somewhat low—even than the corresponding average in the human being.

Moisture, volatile, and extractable substances (c/a)

In oven-dried bone the average was 9.25 per cent.

In alcohol-extracted and oven-dried bone the average was 11.01 per cent.

In ether-extracted and oven-dried bone the average was 11.60 per cent.

*Relation of fixed organic substance to the extracted and dried bone
(e/b)*

In oven-dried bone the average was 32.75 per cent.

In alcohol-extracted and oven-dried bone the average was 33.84 per cent.

In ether-extracted and oven-dried bone the average was 32.83 per cent.

*Relation of fixed organic substance in extracted bone to green
bone (e/a)*

In oven-dried bone the average was 29.77 per cent.

In alcohol-extracted and oven-dried bone the average was 30.22 per cent.

In ether-extracted and oven-dried bone the average was 29.72 per cent.

Having compared the results obtained by the different experimental methods, it will now be of interest to consider the determinations from another standpoint, according to the following tables: Here only the adult human results and cat and rabbit will be considered.

	<i>per cent</i>
Average in green bone at 20 to 60 years	40.75
Average in green bone at 61 to 90 years	42.32
Average in green bone in rabbit	38.90
Average in green bone in cat	39.85

Moisture and extractable substance (c/a)

Average in oven-dried bone 20 to 60 years	8.44
Average in oven-dried bone 61 to 90 years	10.18
Average in oven-dried bone, rabbit	9.89
Average in oven-dried bone, cat	9.25
Average in alcohol-extracted bone, 20 to 60 years	8.46
Average in alcohol-extracted bone, 61 to 90 years	9.01
Average in alcohol-extracted bone, rabbit	11.01
Average in alcohol-extracted bone, cat	10.84
Average in ether-extracted bone, 20 to 60 years	9.27
Average in ether-extracted bone, 61 to 90 years	10.87
Average in ether-extracted bone, rabbit	11.60
Average in ether-extracted bone, cat	10.26

Relation of fixed organic substance to dried and extracted bone (e/b)

Average in oven-dried bone, 20 to 60 years	35.17
Average in oven-dried bone, 61 to 90 years	36.22
Average in oven-dried bone, rabbit	31.60
Average in oven-dried bone, cat	32.75
Average in alcohol-extracted bone, 20 to 60 years	35.07
Average in alcohol-extracted bone, 61 to 90 years	37.00
Average in alcohol-extracted bone, rabbit	30.58
Average in alcohol-extracted bone, cat	33.84
Average in ether-extracted bone, 20 to 60 years	34.16
Average in ether-extracted bone, 61 to 90 years	34.83
Average in ether-extracted bone, rabbit	33.01
Average in ether-extracted bone, cat	32.83

Relation of fixed organic substance to the green bone (e/a)

Average in oven-dried bone, 20 to 60 years	32.23
Average in oven-dried bone, 61 to 90 years	33.15
Average in oven-dried bone, rabbit	28.56
Average in oven-dried bone, cat	29.77
Average in alcohol-extracted bone, 20 to 60 years	32.66
Average in alcohol-extracted bone, 61 to 90 years	32.67
Average in alcohol-extracted bone, rabbit	27.16
Average in alcohol-extracted bone, cat	30.22
Average in ether-extracted bone, 20 to 60 years	31.34
Average in ether-extracted bone, 61 to 90 years	30.82
Average in ether-extracted bone, rabbit	28.82
Average in ether-extracted bone, cat	29.72

Green weight (f/a)

In this table the average amount of organic substance and moisture in green bone (between 20 and 60 years) is 40.75 per cent. This is 6 per cent to 7 per cent higher than that given in text-books. Of course we must admit that this includes moisture and volatile substance and perhaps a very small amount of fat or oil that could not be removed by mere physical means. The specimens were prepared as carefully as possible, and the writer feels that the bone so prepared is standard for all green-weight determinations. The question naturally arises, "Should we include moisture and volatile substances in the per cent of organic material?" Just where to draw the line seems impossible to determine. The small quantities of fat and marrow in

perimedullary haversian canals cannot be removed in this method and they are counted in as part of the organic constituent, and yet they should not be and we are helpless in any effort to remove them.

Again in comparing the results at the prime and after, we find that in the latter instance the average is 42.32 per cent—somewhat higher than in the preceding. This argues for a slightly smaller inorganic content and would argue in favor of the reduction of inorganic as being a factor in the readiness with which the bones of the old fracture made only slight strains. Although the reduction is not so great as to give positive assurance of this, still we must take it into consideration as a possibility. More determinations along this line will be made as the material presents itself.

In comparing the results in the human and in the rabbit (two-thirds grown) we note that in the latter the average percentage of organic material is 38.90 per cent. This indicates immaturity in development and would compare favorably with like stages in the human being, no doubt. Unfortunately, no material was obtainable in individuals from birth to 20 years. From the closeness in the averages, no doubt the per cents in the adult rabbit came very close to that of the human being—showing a close relation in mammals apparently.

In the adult cat the percentage was 39.85—less than 1 per cent below that of the human. If as many determinations had been made in the cat as in connection with human bone, the writer believes that the difference would have been even less, indicating a close relationship in mammals with reference to the composition of compact bones.

Moisture and volatile and extractable substance (c/a)

In this table the average moisture in compact bone in those 20 to 60 years of age is given as 8.44 per cent.

The temperature (52°C.) is not sufficiently high to cause any decomposition, nor was it employed long enough to cause any untoward effects upon the above. Whether anything else

besides moisture is driven off or not was not determined, as that was beyond the province of this paper. This amount of moisture strikes one as quite high, yet when we consider that all of our organs and tissues are principally water (mainly combined) we should not be surprised at these results. It is variable in different individuals, as the detailed results in the earlier tables show. If this be not considered normal organic content, but be subtracted from the green weight, then the average, as will be seen later, gives what is normally called 'fixed organic substance.'

Between the ages of 61 and 90 the percentage of moisture, etc., is 10.18 per cent, and as we proceed in this analysis we will note that the increase in these ages is constant. The difference between these age groups is only 1.75, yet that is a definite increase.

In the rabbit the average moisture, etc., is 9.89 per cent, and this does not seem excessive for the proportionate growth, as the percentage in the young is greater than in the adults and then in old age seems to increase again.

In the cat the amount of moisture is 9.25—nearly 0.75 per cent greater than in the adult man. This is possibly a normal condition for that animal.

In the alcohol-extracted bone the percentage is almost identical with that of oven-dried bone, being only 8.46 per cent. It seems that compact bone prepared in the way for these determinations contains very little alcohol-soluble substance. In those of 61 to 90 years the percentage is only 9.01—below the weight of ordinary moisture. This variation cannot be accounted for.

In the rabbit the average is 11.01 per cent and in the cat 10.84 per cent, showing over 1 per cent increase in both instances. Again, the relatively higher percentage in the rabbit indicates a more youthful state.

The ether-extractable substance is greater. In the human bone 20 to 60 years the average is 9.27—a material increase over moisture and alcoholic extract. This increase is constant in all. In the rabbit the average is 11.60 per cent, in the cat 10.26 per cent. In all of these forms of animals there is a higher percentage

of ether-extractable substance, and this probably is the fat in the perimedullary haversian canals. This amount of ether-extractable substance is material in relation with total amount of moisture, but in reference to the total amount of organic substance it is not so marked. We must, however, realize that there is some ether-soluble organic material even in the compact bone and something which is difficult to classify, so the question arises, "Should it be included in the organic constituent or not?"

Relation of fixed organic substance to the dried and extractable bone (e/b)

As has been seen, the percentage of organic substance in green bone includes the moisture and soluble substances, and this naturally increased that percentage. Should they be included as organic constituents or excluded? If we exclude them, then we have remaining the real fixed organic substance and then we can deduce two different answers: 1) The relation of this fixed organic substance to the weight of the extracted bone (green bone less moisture and extracted substance e/b): The relation to the original green weight (without the removal of any moisture, etc., e/a).

Of course, the percentage in reference to the extracted bone weight (e/b) will be the greater and we find that the percentage in those 20 to 60 years is 35.17 per cent. This is a per cent or so above that given in text-books, but we are not told the method of determination, and the writer for one, does not believe that this was the method used. In those of 61 to 90 years the average is 36.72 per cent—again higher than in the earlier years. In the rabbit it is 31.60 and in the cat it is 32.75 per cent—considerably lower than in the human bone.

In the alcohol-extracted bone in those 21 to 60 years, the average is 35.07 per cent, while in those 61 to 90 years, the average is 37 per cent. Again, this determination shows less alcoholic extract than mere moisture as in the last table. This seems to indicate that alcohol may fix and render insoluble or non-volatile

some substances in the bone that are volatile with mere heat or soluble in ether. So the figures would argue at any rate. In the rabbit the average is 30.58 per cent and in the cat 33.84 per cent. In the ether-extracted bone, 20 to 60 years, the average is 34.16 per cent of organic substance as compared with the extracted weight of the bone. At 61 to 90 years the average is 34.84 per cent in both instances—a drop over the corresponding moisture and alcohol-extracted percentages. This is in keeping with the relatively higher amount of ether-extractable material, and should be so. In the rabbit the average is 33.01 per cent and in the cat 32.83 per cent. These are properly proportionate results.

Relation of the fixed organic substance to the green weight (e/a)

In these results the percentages should be lower than in the preceding, as we start with a higher original weight, including therein all moisture and extractable substances. In those 20 to 60 years of age the average is 32.23 per cent and in those 61 to 90 years the average is 33.15 per cent.

In the rabbit the average is 28.56 per cent and in the cat 29.77 per cent.

These percentages could all have been got by merely subtracting the percentage of moisture from the original average in green bone, but this was not done, but each was determined from the resulting weights, so in some instances the sum of the moisture percentage and this last per cent (e/a) does not always give the exact per cent of organic substance in green bone, but the per cent according to the figures obtained. We note in all these last determinations a constant and normal difference from the preceding determination.

In the alcohol-extracted bone, 20 to 60 years, the average is 32.66 per cent and in those of 61 to 90 years the average is 32.67 per cent—almost identical. In the rabbit the average is 27.16 per cent and in the cat 30.22 per cent.

In ether-extracted bone, 20 to 60 years, the average is 31.34 per cent and from 61 to 90 the average is 30.82 per cent. In

the rabbit the average is 28.82 per cent and in the cat 29.22 per cent. These are all consistent variations and will be noted by consulting the corresponding table in the other methods of determination.

CONCLUSIONS

Five methods for the determination of organic content of bone have been here given. Of these the ordinary method of cleaning (maceration) should be avoided, as results cannot be consistent owing to the irregularity with which bones of the same individual degrease.

All of the other four methods are constant and reliable, and every one may be considered as a standard. Various points for discussion arise. Should the moisture be included in the organic content? Should any alcohol- or ether-soluble substance be included in the organic content?

If we examine the tables, we find that the only percentages close to those given in text-books are those obtained in ether-extracted bone and in relation to the weight of the bone after extraction by the ether (e/b).

This may have been the method employed by the early chemists. It was employed here to try to determine the nearest to the text-book percentages. This determination (e/b) may seem unfair because it first removes the soluble and volatile substances and takes the weight of this dried bone as a standard to determine the fixed organic substance. It does not seem fair, but it discriminates against volatile and extractable substance. It can be argued that this moisture and this soluble material are real organic contents and that they should be so included just as in making an assay of ore, the whole sample is crushed and mixed for test and not just certain parts selected. In the latter instance any desired results could be obtained and these would not be the true or fair results. If the moisture and soluble material be considered organic content, then our text-books are incorrect to the extent of 6 per cent to 7 per cent. If, on the other hand, they are excluded and the percentage still be

determined in the green weight, then the text-book percentage is 2 to 3 per cent too high. Even in oven-dried bone the percentage in reference to the dried weight is still too high—37.15 (20 to 60 years) and 36.22 (61 to 90 years).

The writer would be inclined to consider the green weight the proper one for starting. After the bone has been freed of all periosteum and cancellous tissue and surface oil, its weight should be the one used, and the ultimate results would come under the determinations f/a , in which the average is 40.75 per cent (20 to 60 years) and 42.32 per cent (61 to 90 years).

The writer would like to have the opinion of others along this line so as to establish a standard for determination of the organic content of compact bone. Further studies in this connection are under way.

Resumen por el autor; Eben J. Carey.

Estudios sobre la estructura y función del intestino delgado.

- I. La arquitectura helicoidal del intestino delgado.
- II. La acción espiral del intestino delgado.

La capa muscular interna del intestino delgado es una lámina continúa arrollada en espiral apretada. Una vuelta completa de espiral tiene lugar en cada 0.5 a 1 mm. de longitud, o menos. La capa muscular externa forma una espiral alargada, que termina una vuelta completa en una extensión de unos 200 a 500 mm. o más. La submucosa está compuesta de fibrillas de tejido conjuntivo que forman una espiral interna apretada y otra externa más laxa. La interna efectúa una vuelta completa en 0.5 a 1 mm. o menos, la externa en cada 4 a 10 mm. La capa muscular interna, por consiguiente, está arrollada en espiral apretada, la externa en espiral laxa.

La diferencia en la velocidad de la progresión translatória de las dos ondas de contracción depende de esta disposición en espiral. La onda que marcha a lo largo del grupo interno de fibras toma un curso rotatorio, mientras que la que camina a lo largo de las fibras externas sigue una dirección más translatória hasta alcanzar su punto de destino. Por consiguiente, la contracción de la capa muscular interna, más fuerte, seguirá inevitablemente a la de la capa externa. La disposición de las capas musculares intestinales explica claramente el fenómeno de la contracción cefálica y la dilatación caudal durante la diastalsis, sin necesitar invocar la ayuda de vías nerviosas hipotéticas. La peristalsis, por consiguiente, es un fenómeno de doble contracción producido por la velocidad diferencial del avance translatório de las dos ondas de contracción en las capas musculares externa e interna, respectivamente. Estas conclusiones se basan en experimentos en los cuales el autor separó del intestino vivo las capas externa e interna.

STUDIES ON THE STRUCTURE AND FUNCTION OF THE SMALL INTESTINE

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TWENTY-TWO FIGURES

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1. THE HELICOIDAL ARCHITECTONICS OF THE SMALL INTESTINE

Introduction

The current literature and text-books of anatomy and the related medical sciences describe the two muscular coats of the small intestine as "inner circular and outer longitudinal." The inner muscular coat is conceived, therefore, as forming a tube composed of discrete muscular rings with a certain degree of connection. Such a conception is a faulty anatomical heirloom and, due to its apparent truth by a mere cursory microscopical observation, is accepted by the present generation as an assimilated fact. This erroneous idea arose with the inception of the microscope and has since been accepted unchallenged. Mall ('96) demonstrated the spiral nature of the submucosa twenty-

five years ago, but this important observation has remained as an obscure isolated report. The fact that the musculature possesses also a spiral architectonic did not occur to him.

The truth in regard to the form of a muscular structure is impossible when the evidence rests on microscopic observations of single sections. Painstaking reconstructions of serial sections may be an aid, but only when it is impossible to make gross dissections. The pitfall presented by the microscope reminds one of the following wise admonition given by John Goodsir ('46) to his freshman class in anatomy, "As soon would the astronomer place the telescope in the hands of his pupil and request him to interpret the sinuous lines by which the orbits of the planets are projected on the apparent surface of the hollow sphere, before he has acquired steady ideas of astronomical forms and motions by preparatory study, as would the judicious teacher of anatomy suggest the examination of objects by the microscope before strict anatomical ideas of form and relation had been acquired by the study of the gross specimens of the bones, viscera, muscles and blood vessels."

Studies on the interaction of the intestinal components (Carey, '19, '20 a; '20 b; '20 c) during the genesis of these two muscle coats reveal the following facts: The primitive gut presents two zones of differential rates of growth. There is an inner epithelial tube, growing in the manner of a left-handed spiral, of accelerated growth. Secondly, there is the outer mesenchyme, of retarded growth, from which the two muscle coats are derived. During the period of rapid transverse growth of the inner tube, the inner muscle coat is seen to become progressively drawn out by traction. This coat, then, by compression, restricts the diametrical growth of the entodermal tube. The planes of mitosis shift from a parallel to a transverse position as regards the long axis of the gut and the subsequent growth of the inner tube is relatively more rapid in length than in width. During this rapid longitudinal growth, the outer mesenchymal cells are drawn out by traction, revealing the first step in histogenesis, namely, an elongation of the myoblastic cell.

Since the tension in the mesenchyme corresponds to the spiral lines of the dominant growth-force, of the entodermal epithelial tube, the mature structure, as the muscular resultant of this interaction of growth and resistance, under no circumstances could possess a circula form. This assertion is based purely on embryological evidence. The confirmation of this evidence led to the following observations of gross dissections.

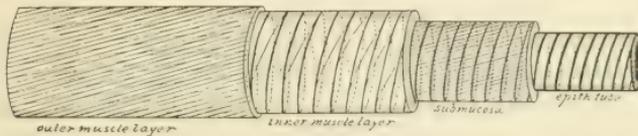


Fig. 1 Schema of the left-handed helicoidal structure of the small intestine based on dissections. The mitosis of the epithelial tube follows the path of a left-handed helix. Mucous folds are directed the same as the mitotic paths. This is seen in the valvulae conniventes which primarily form left-handed interdigitating spirals. This is readily demonstrated by everting the duodenum or jejunum. The submucous connective tissue forms inner close spirals and outer elongated spirals. This same arrangement holds for the muscular coats. The fasciculi of the muscular coats and the fibers of the submucous coat are connected by anastomosing elements, respectively. Muscular action based on this arrangement, therefore, is in the manner of a screw corresponding to the direction of the muscular fibers.

Direct observations on the helicoidal structure of the muscle coats of the small intestine

The small intestine of the pig is everted over a test-tube. By making a longitudinal incision through the mucosa and the submucosa these coats are then easily dissected from the inner muscular layer. Once the inner muscular layer is thoroughly cleaned of submucous and areolar tissue, a single smooth muscular fasciculus may be easily isolated and traced around the test-tube. By this means one may demonstrate incontestably that the fasciculi of the inner smooth muscle coat are wound closely in a compact spiral (fig. 1).

The main point to keep in mind in regard to this arrangement in the adult is that it reflects its embryonic origin. The muscle is derived from an embryonic syncytium which had been whorled

into a vortex. Consequently, there is bound to be intercommunications found between the spirally arranged fasciculi in the mature state.

In many instances one may trace a well-developed smooth muscle fasciculus in the duodenum or lower ileum near the cecum as a definite entity through ten or fifteen turns. This demonstrates that the inner intestinal muscular coat is not composed of discrete contiguous rings, but is a continuous syncytial muscular layer spirally wound anticlockwise.

By inserting a test-tube in the lumen of the intestine and dissecting off the serosa it may be demonstrated that the fasciculi of the outer coat do not run a straight longitudinal course, but run slightly in an oblique direction. In many instances a definite fasciculus or group of fasciculi may be traced from one side of the intestine to the other. There is a tendency to obliterate the spiral nature of the outer coat due to the torsional reaction of the intestine. This is manifested by the formation of loops which tend to counteract the strain to which the intestine is subjected in the spiral development of the entodermal epithelial tube. At the same time, this reaction tends also to counteract the spiral course of the outer layer.

The inner layer makes a complete turn in every 0.5 to 1 mm. or less; the outer layer makes a turn in every 200 to 500 mm. The definite obliquity of this outer layer is best detected after cleaning thoroughly along the line of the mesenteric attachment. It is then clearly demonstrable that the outer fasciculi do not run in a longitudinal course parallel to this landmark, but at an acute angle from right to left (fig. 1).

The spiral nature of the connective tissue of the submucosa may be demonstrated by inserting a test-tube in the lumen of the small intestine and dissecting off the serosa and musculosa. By allowing the submucosa and mucosa to incubate at 37°C, autolysis will occur, leaving the fibers intact. As desiccation proceeds, the fibers adhere to the test-tube, forming two definite spiral arrangements. There is an inner close spiral and an outer elongated spiral both wound anticlockwise or as a left-handed helix.

Mall ('96) detected the spiral nature of the submucosa, but described it as composed of fibrillae running in opposite directions, forming a spiral lattice-work. The observation of the spiral nature is correct, but the fibrils are arranged in two sets running in the same direction. The inner spiral is wound closely, the outer is more elongated. The latter makes one complete turn every 4 to 10 mm. the former in every 0.5 to 1 mm. or less (fig. 1).

Conclusions

1. The inner muscle coat of the small intestine is not composed of circular or annular rings contiguously placed, but is a continuous muscular sheet wound into a close helix. One complete turn is made in every 0.5 to 1 mm. or less.

2. The outer muscle coat of the small intestine is not composed of longitudinal fibers parallel to the long axis, but of elongated fibers, at an acute angle to the long axis of the intestine, which tend to pursue a spiral course. This spiral character is obliterated in many places, due to the torsional reaction of the intestine. Those which are spirally disposed make a complete turn in every 200 to 500 mm. or more.

3. The submucosa is composed of connective-tissue fibrils forming an inner close and an outer elongated spiral. The inner makes one complete turn in every 0.5 to 1 mm. or less, the outer in every 4 to 10 mm.

4. Therefore, the structure surrounding the epithelial tube of the intestine in the mature state possesses a spiral nature arranged as a left-handed helix with an inner close and an outer elongated set of spirals in both the submucosa and musculosa.

5. The valvulae conniventes of the duodenal epithelium are arranged as interdigitating spirals.

6. In the adult, consequently, confirmatory evidence is presented substantiating the thesis that the left-handed helicoidal growth of the epithelial tube creates a corresponding mesenchymal vortex. From the latter the spiral structure of the submucosa and musculosa is developed. This competition between the accelerated growth of the entodermal tube and the

retarded growth of the surrounding mesenchyme leading to a formative interaction of differential growth has been overlooked as a factor in histogenesis.

2. OBSERVATIONS ON THE SCREW-LIKE ACTION OF THE SMALL INTESTINE

Introduction

Since the direction of muscular contraction is dependent on the form and arrangement of the muscular fasciculi, it will be profitable to study intestinal action in the light of the spiral architectonics of the musculature. Form and function are inseparable; consequently, a contribution to anatomical structure is an aid to understanding its related function.

The study of the reciprocal elongating actions of the two muscle coats of the intestine presents one of the most confusing chapters in the entire literature of anatomy and physiology. Prior to the graphic records obtained by Bayliss and Starling ('99 a) it was thought that the outer and inner muscle layers contracted alternately by Ludwig ('61), Englemann ('71), Nothnagel ('82), Luderitz ('89), Mall ('96 a). Bayliss and Starling proved that these muscle layers contract simultaneously. But they interpreted their classical records by stating that contraction takes place in the two muscular layers in the same place at the same time. Although contractions begin in the two layers at the same position at the same time, the typical peristaltic resultant is at different locations in successive units of time. This will be proved to be inevitable because of the arrangement of the two sets of muscular fasciculi.

There are three main types of movement seen in the small intestine. First, there is a rapid vermicular wave. This is primarily seen after death or in animal experimentation. It progresses through the course of the small intestine in about a minute. It is considered by Mall ('96 b) as being more pathological than physiological. Second, there is the slow-moving peristaltic wave called by Cannon ('12 a) diastalsis. This normal wave propels the food through the small intestine in three to five hours. Third,

there is seen rhythmic segmentation which may or may not be combined with peristalsis. By combination of the two movements there is a slow advance of the food combined with definite constrictions of the intestinal contents. There is a rhythmic repetition of the segmentation. Normally, the wave progresses from above downward.

The slow peristaltic wave presents a marked constriction above and a marked dilatation below the stimulated area. This phenomenon has been called by Bayliss and Starling ('99 b), the 'law of the intestine.' They state this law as follows: "Local stimulation of the gut produces excitation above and inhibition below the excited spot. These effects are dependent on the activity of the local nervous mechanism."

That Bayliss and Starling ('99 a) exclude absolutely the fundamental arrangement of the two muscular layers as shedding light on the interpretation of the peristaltic wave is seen in the following statement (p. 114): "We cannot imagine any muscle fibre or collection of muscle fibres which would relax on one side and contract on the other side of an excited point."

Bayliss and Starling ('99 d) invoke, therefore, the aid of hypothetical nerve paths to explain the apparent paradox as follows (p. 115): "The different time relations of the two reflex actions would lead one to guess that the system is composed of long paths which conduct inhibitory impulses downwards, and short paths which carry augmentor impulses from one cell station to another in an upward direction." After twenty years no objective histological evidence has been presented confirming the nerve paths assumed by Bayliss and Starling in their interpretation of the peristaltic complex.

Magnus ('04) proved that Meissner's (submucous) plexus is not involved in the production of the slow peristaltic wave. It was modified or inhibited by sectioning the vagus nerve and by the use of nicotine. Consequently, the true peristalsis Magnus considered as due to Auerbach's (myenteric) plexus. Cannon ('12 b) therefore called the 'law of the intestine' the myenteric reflex.

That Mall ('96 c) surmised the active influence of the muscular arrangement in the propulsion of food through the intestine is seen in the following:

"It therefore seems that there are at least two forces which move a body through the intestine. 1. The body as an irritant causes the circular muscles above it to contract, which in turn pushes the body down. A new portion of the mucosa is irritated, which causes a new contraction and so on. 2. At the same time a sucking force, due to an active dilatation below the body, may have a tendency to drag it down."

Mall, however, did not reveal the factor causing the active dilatation. Cannon ('02 c) pointed out this discrepancy in Mall's argument, as follows: "In what manner an active dilatation of the intestinal wall may occur so as to produce a 'sucking force,' Mall does not wholly make clear." At the same time, Cannon also refrained from clearing up the question at issue.

The object of this paper, therefore, is to report results of experiments and of direct observations of the musculature of the small intestine in the light of the spiral architectonics of the two muscle layers. *The law of the intestine or myenteric reflex is proved to be an inevitable duplex contraction phenomenon based on the fact that the inner coat is a close spiral and the outer an elongated spiral or longitudinal muscle layer. This arrangement of the two sets of muscles results in a differential rate of translatory progression of the two contraction waves in the outer and inner coat, respectively. The wave in the outer coat will pursue a greater longitudinal distance per unit of time than that in the inner coat, because the wave in the inner coat pursues a more rotary course, to reach the same destination, than that in the outer coat.*

Experimental observations

A. *Experimental excision of the inner close spiral muscular coat of the intestine.* The following observations are true for the dog, cat, pig, sheep, and cow. About a foot of the small intestine was inverted under warm oxygenated Ringer's solution soon after the animal was killed. The mucosa and submucosa were

quickly dissected, making an annular gap of about 1 inch. By making two longitudinal incisions, one along the mesenteric and the other opposite the mesenteric attachments through the inner spiral coat, the latter may be quickly dissected from the outer coat. The gut is then reinverted to its normal position (fig. 2). After a quick, clean dissection and if the excitability of the musculature is still present, a satisfactory demonstration may be made (figs. 2 and 3).

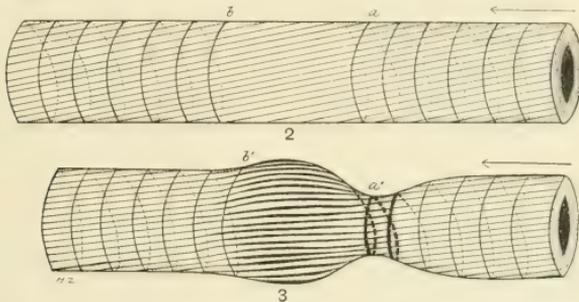


Fig. 2 This represents a segment of the gut from which the inner spiral coat was excised between *a* and *b*. The arrow is directed caudad.

Fig. 3 This represents a peristaltic wave in relation to the area of excision of the inner close spiral muscle coat. *a'* designates the cephalic constriction. From *a'* to *b'* depicts the caudal dilatation. This bulging immediately evident is due to the outer elongated muscle layer. A stimulus applied to the muscle layers of the gut will produce two effects due to the different directions of the muscular fasciuli. The reacting contraction wave of the inner coat will pursue a rotary course, whereas that of the outer coat will pursue a translatory course. Therefore, the cephalic constriction produced by the contraction of the stronger inner coat is bound to trail the caudal dilatation produced by the outer coat.

Stimulating by pinching or by inserting a vaselinated cotton bolus or by means of the induction current, a peristaltic wave may be started above the annular excised area. The dilatation is immediately detected below the stimulated spot and constriction above. It is definitely evident by direct observation that the outer muscle coat contracts in advance of the inner muscle coat as the constriction nears the annular area of excision. The constriction due to the stronger inner coat is cephalad or proximal to the area of excision, at the same time that the outer muscle

coat contracts caudad or distal approximating the edges of the excised region a' to b'' (fig. 3).

Simultaneous with the approximation of the edges of the excised area there is a tendency to bulging. This bulges primarily outward, but may bulge inward. This is comparable to the bulge of the fibrils of a twisted string when relieved of the torsion. The fibrils as they unwind tend to give a characteristic spindle-shaped bulging. That a shortening of the outer coat takes place in advance of that of the inner coat is, therefore, objectively evident.

This experiment proves that the active contraction of the outer coat is a definite factor in the production of the caudal dilatation seen in the intestinal peristalsis. The constricted, cephalic component is usually blocked upon reaching the excised region, in a few instances a slight propagation was detected distal to the excised region, showing that the elongation of the inner coat produced by the contracting outer coat had taken place. If only a 5- or 10-mm. annular gap is made, the wave is not as frequently blocked as when 30 or 40 mm. are excised.

The inner and outer coats act, therefore, as reciprocal elongators during the propagation of the peristaltic wave. The integrity of each muscle is necessary for normal peristalsis. Normal peristalsis is a double contraction wave. The wave of contraction of the inner coat, following the path of a close spiral, trails that of the outer coat which follows the path of an elongated spiral. The contraction of the inner coat causes the cephalic constriction, that of the outer coat the caudal dilatation.

B. Experimental excision of the outer elongated spiral muscle coat. The small intestines of the same animals are used as in the foregoing experiment. The serosa and outer muscular layer are excised under warm oxygenated Ringer's solution. Two circular incisions of varying distances are made. The incisions are connected by means of two longitudinal incisions, one along the mesenteric and one opposite the mesenteric attachment. The incisions extend to the circular coat. A quick dissection of the serosa and outer muscle layer is now made, leaving the inner muscle layer between the circular incision (fig. 4).

By now stimulating the intestinal tube as before, the typical cephalic constriction and caudal dilatation of the peristaltic wave are started down the tube in the normal direction caudad. In figure 5 the peristalsis is seen a few millimeters from the ex-

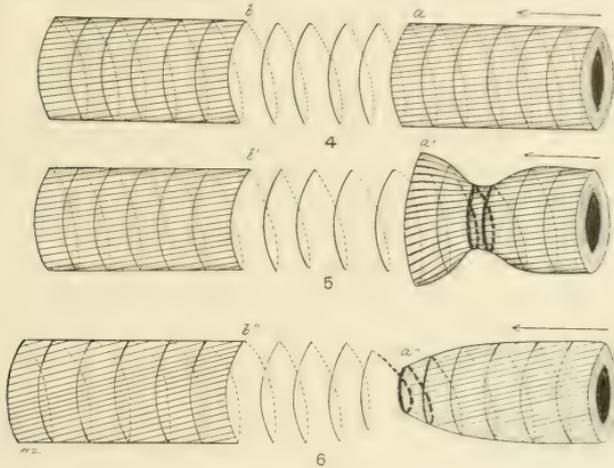


Fig. 4 Diagram of gut from which the outer muscular coat was excised from *a* to *b*.

Fig. 5 Diagram of peristaltic wave bordering the area in which the outer muscle layer is absent. Note especially that the caudal dilatation is shortened and totally lacking over the area in which the outer muscle layer was excised.

Fig. 6 The cephalic constriction of the peristaltic wave borders the upper aspect of the area in which the outer muscle layer is absent. From *a''* to *b''* no dilatation is seen. This gap acts as an effective barrier to the propagation of the peristaltic wave if it extends 30 to 40 mm. in length. A greatly diminished wave was seen once to extend caudad in which the gap was only 5 mm. Such a gap ordinarily acts as a barrier if no fluid content is within the lumen of the intestine. At *a''* rhythmic contractions may occur for some time before the gut musculature comes to tonic equilibrium at this point. That the two muscle layers are reciprocal elongators as peristalsis advances is evident.

sected zone with a foreshortened caudal dilatation. As soon as the constriction reaches the edge of the operated region (fig. 6) no caudal dilatation is detected. The outer coat is absent at the gap and coincident with the absence of continuity of the outer coat there is an absence of the typical caudal dilatation.

If the outer coat is exsected for only 5 mm., an abortive continuation of peristalsis may infrequently be detected. But primarily the lack of anatomical integrity of the outer coat acted as an efficient block to the propagation of peristalsis. Frequently a rhythmic series of contractions similar to rhythmic segmentation is detected at the cephalic aspect of the gap.

Interpretation

These two experiments prove that the anatomical continuity of the outer and inner muscular coats is necessary for the conduction of the normal peristaltic wave. When the inner coat is exsected the caudal dilatation is seen over the operated region concomitant with the cephalic constriction a few centimeters above. This proves that the inner stronger muscle is the cause of the cephalic constriction and that the caudal dilatation is due to the outer longitudinal coat.

When the outer longitudinal coat is dissected off, there is a progressive shortening of the caudal dilatation as peristalsis nears the exsected region. As the constriction nears this zone there is a progressive shortening of the caudal dilatation until it is totally absent as the constriction borders the area of operation. This confirms the conclusions reached in the first experiment.

The normal peristaltic wave is an exaggeration of the waves of rhythmic segmentation. The former presents a zone of constriction 6 to 8 cm. long in the dog's gut, whereas the latter presents constricted zones only 1 cm. long. The peristaltic wave or diastalsis has a longer constricted zone, due to the fact that a greater resistance is presented. It is the duplex contraction wave which overcomes the resistance in propelling the contents of the small intestine caudad.

On the other hand, the waves of rhythmic segmentation knead or segment the intestinal contents. A less resistance is presented, consequently a shorter constriction is found. In order that rhythmic segmentation may be effective, about 1 to 2 cm. of intestinal musculature is necessary. What the determinants are that cause either the diastaltic or rhythmic waves of segmentations are not, as yet, determined.

Cannon ('12 d) found that the "normal gastric peristalsis does not require the reflex mechanism for the waves sweep from the pulsatile source to the pylorus, in an orderly manner, after the myenteric plexus has been completely interrupted by a half dozen incisions encircling the stomach." In the small intestine Cannon ('12 e) made encircling incisions through the musculosa to the submucosa, "at intervals varying between 1.5 and 2 cm. throughout the first 45 cm. of the small intestine." He found that segmentation is present in the operated region and that peristalsis is stopped.

The inference, however, that "peristalsis of the small intestine, therefore, unlike that of the stomach, is seriously interfered with by division of the myenteric plexus," Cannon ('12 f) is not to be derived from these experiments. As regards the small intestine, the intervals between the encircling incisions are too narrow for effective muscular waves of normal peristalsis. As Cannon ('12 g) observes in the cat, the trough of the wave is 4 to 5 cm. long. Those encircling incisions have intervals, the longest of which are only 2 cm.; therefore, the musculature has been effectively put out of commission in order to manifest diastalsis, but its integrity is still intact to exhibit the shorter waves of segmentation. I repeated Cannon's experiments on the small intestine of the cat, cutting encircling incisions at intervals of 10 to 15 cm., and found that the normal peristaltic wave was present and had not been interfered with by division of the myenteric plexus.

If it is remembered that diastalsis is the wave of propulsion and that segmentation is the wave of kneading, the difference of resistance to be overcome by each is self-evident. Diastalsis needs a greater extent of the gut for effective muscular action than that of segmentation. If the anatomical continuity of the optimum extent of the gut, necessary for diastalsis is interfered with, it cannot be elicited. The integrity of the muscle was so interfered with in Cannon's experiments for effective peristaltic action.

This action was not inhibited in the stomach, due to a number of circumstances. First, the short length and greater width of the stomach compared with that of the small intestine. Second,

when the musculature of the stomach contracts between the encircling incisions, a greater action is manifested on account of the greater encircling extent of the fibers as compared with the shorter encircling extent of the fibers of the small intestine. Third, the reaction to muscular contraction in the stomach, therefore, is greater. The fluid contents of the stomach, consequently, will act as a medium for the propagation of the distending wave, causing a stretching and subsequent contraction of the fibers between the next neighboring encircling incisions.

In the small intestine the lessened diameter will not allow for as great a muscular action along 2 cm. linear extent as along a corresponding portion of the stomach. A greater extent of anatomical continuity is necessary, therefore, in the small intestine than in the stomach in order to make a similar comparison of muscular action between these two organs. By making the intervals 10 cm. between the encircling incisions in the small intestine, the peristaltic wave is not inhibited.

The difference in the power of the stomach and small intestine is readily apparent by the following brief consideration of the physical principles of the screw: The screw is a modification of the inclined plane, and the conditions of equilibrium are those which obtain in the case of the plane. The resistance, which is either a weight to be raised or a pressure to be exerted, acts in the direction of the vertical, and the power acts parallel to the base; hence we have $P:R = h:2\pi r$; r being the radius of the cylinder and h the pitch of the screw. (The vertical distance between any two threads of a screw measured parallel to the axis is called the *pitch*.)

The power is usually applied to the screw by means of a lever, as in a bookbinder's press. In the gut the power is derived by muscular contraction and the principle of the screw may be stated to be generally, the *power* of the screw is to the *resistance*, in the same ratio as that of the *pitch* of the screw is to the *circumference* of the apparent circle through which the power acts. This means that, other factors being equal, a greater power would be manifested in a given segment of the stomach than in a corresponding length of the small intestine because of the difference in circumference.

Reciprocal elongation of muscles

The contractile state of muscle, as well as the relaxed, arises from a power inherent in itself. The elongation or stretching superimposed on a muscle in tonic contraction depends on some extrinsic power. Simple relaxation of a contracted muscle is not sufficient to enable it to produce another requisite effect. It is necessary that there should be an elongator equal to the quantity of contraction intended to be produced. No muscle has the power of adequately extending or stretching itself; therefore, there must be an elongator. The elongators are usually muscular, but elastic tissue may serve this function as well as fluids in musculotubular organs, like the bladder.

The reciprocal elongation of muscles is strikingly evident in the intestine. Rhythmic contraction is due to a reciprocal mechanism, each wave is composed of a contraction and an elongation of the inner spiral coat alternating with a contraction and an elongation of the outer elongated spiral or longitudinal muscle coat. At the start the contraction waves of both coats begin together, but, due to the rotary course of the inner wave and the translatory course of the outer wave, the former and stronger one will inevitably trail the latter and weaker one. The outer and inner muscles are reciprocal elongators, therefore, as peristalsis extends through the intestine (figs. 7 and 8).

When the outer and inner muscles are in normal tonic equilibrium (fig. 7) no distortion is evident. As soon as a contraction wave starts the balance is upset. The stronger cephalic constriction (fig. 8) causes an elongation of the outer muscle coat. The wave of the latter follows in the path of the distal region of elongation. The contraction of the outer coat causes an elongation of the inner coat in the region of the caudal dilatation. Subsequently, the contraction wave of the inner coat is seen to occupy the former zone of stretching in the region of the caudal dilatation. There is, therefore, a definite syncopation in the activity of the outer and inner muscle coats as the peristaltic wave travels through the intestine. The muscle coats act as reciprocal elongators, consequently peristalsis progresses for a variable distance through the gut instead of coming to a dead center.

Direct observation of diastalsis

When the stomach is distended with food, a discharge of chyme into the duodenum takes place. Upon dilatation of the pyloric constrictor there is then produced an elongation of the outer and inner muscular coat overlying the bulged area. The reaction to this stimulus of elongation is contraction. The stronger

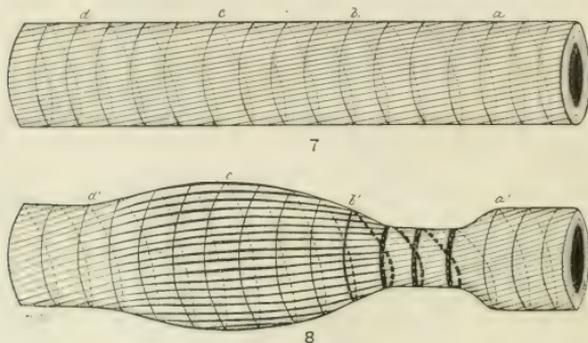


Fig. 7 Diagram of small intestine in which the two spiral muscular layers are in tonic equilibrium.

Fig. 8 Diagram of peristaltic wave. The balance of tonicity between the inner and outer muscle layers is upset. From a' to b' the stronger inner coat upsets the balance by causing the cephalic constriction. In the dog this trough is 6 to 8 cm. long. From b' to d' the outer coat upsets the balance by contracting. This shortens and dilates the gut. The action of the outer muscle layer acts as an effective elongator of the inner layer. This stretching stimulus puts the inner layer in a condition for immediate contraction. The outer muscle layer is put in tension caudad to d' . The contraction wave, therefore, travels progressively down the tube, due to the effective reciprocal stretching of the muscle fibers taking place distal to the contraction wave. The cephalic aspect of the tube is to the right, the caudal aspect to the left of the observer.

inner coat contracts and causes a cephalic constriction. The outer coat contracts and tends to shorten the gut forming the caudal dilatation.

The spiral structure and screw-like action of the intestinal muscles help to explain the normal direction of the peristaltic wave from above downward. Again, the fact that contraction of muscles follows in the wake of a stretching or an elongation

also acts as an effective determinant stimulus. Since the cephalic aspect of the small intestine is the first part stretched by the chyme flowing through the pyloric valve caudad, this region will be the first to respond to the elongating stimulus by contraction.

This muscular action propels the food still further caudad. Successive areas are progressively elongated. They respond in turn by contraction. This alternation of stretching and subsequent contraction of the muscle continues on down the small intestine. The effective stimulus of elongation due to the intestinal contents together with the continuous spiral nature of the musculature readily explains the normal peristalsis as a wave progressing from above downward.

Once this direction of action is implanted in the spiral muscles, it tends always to react by screwing in the same direction as that which the food would normally take. There seems to be a physiological polarity. Every section cut from the gut tends to keep the polarity of cephalad and caudad that it originally possessed while in the intact gut. This is comparable to the analogy cited by Loeb ('12) with regard to the magnet, as follows:

"If a magnet is broken into pieces, every piece has its north pole on that side which in the unbroken magnet was directed toward the north. Likewise, there are animals every piece of which produces, at either end, that organ toward which it was directed in the normal condition. We may speak in such cases of polarization."

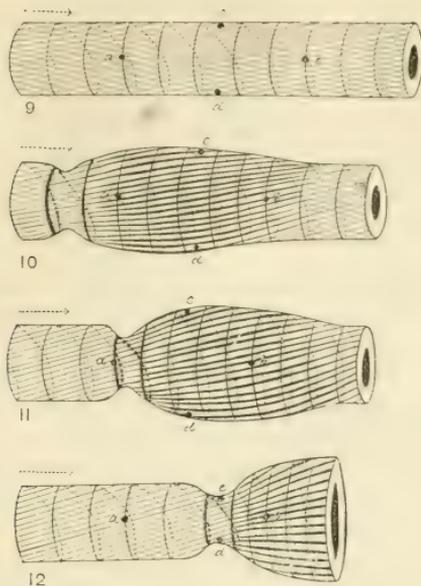
This would explain the stasis observed by Mall ('96 d) in experiment upon the reversal of the gut. The reversed segment by maintaining the original screw-like action that it had in the intact gut would now counteract a wave of peristalsis progressing downward. This is comparable to two screws opposed in action.

There is, however, eventually a tendency for the reversed segment of the gut to become adapted to its abnormal environment. It transmits subsequently the wave of peristalsis from above downward directly opposite to that which it did previously. This reversed conveyance of food was seen by Kelling ('00), Enderlen and Hess ('01), Beer and Eggers ('07), McClure and Derge ('07).

One fact to be emphasized finally in regard to the movement of the intestine is the left-handed screw action of the spiral musculature. A section of the pig's intestine 6 inches in length was excised under warm saline solution. By inserting a vaselinated cotton bolus in the cephalic opening of the gut, a strong cephalic, anemic constriction is elicited. In a short time the bolus is expelled at the caudal orifice of the intestine. Inspection of the external surface of the bolus reveals a left-handed spiral arrangement of the vaseline coat. The same may be observed on a bolus of warm agar-agar or soft paraffin. The actions of the intestinal spiral muscles are, therefore, in the manner of a left-handed screw in the pig's intestine. These observations were made on the killing floor in the Cudahy abattoir, South Omaha.

The explanation of Bayliss and Starling's graphic record is schematized in figures 9, 10, 11, 12, 16, 17, 18, 19, 20, 21, 22. At each rhythmic contraction of the inner coat a contraction wave is started through the outer muscle coat. To detect the contraction waves in each muscle coat two enterographs are placed at right angles to each other. A shortening of the distance between *a* and *b* (fig. 9) is indicative of contraction in the outer layer of muscle. A shortening of the distance between *c* and *d* (fig. 9) indicates a contraction of the inner muscle coat. As the cephalic constriction progresses caudad toward the registering points, rhythmic contractions of the outer muscular layer corresponding to each contraction of the inner coat are detected in the area of caudal dilatation. This is considered by Bayliss and Starling as due to inhibitory nerve impulses which prevent contraction of the inner muscle. But it is due, however, to the contraction of the outer coat which throws the balance of tonic equilibrium between the two muscle layers in favor of the contracting outer coat, thus causing an active dilatation. This enlargement is not due to a passive relaxation or inhibition of the tonicity of the inner muscle layer. This was definitely proved in the two experiments cited above.

When the zone of cephalic constriction causes a graphic registration of the approximating points *c* and *d*, there is also a shortening between *b* and *a*. Both layers will register simultaneous



Figs. 9 to 12 These figures are explanations, based on the spiral architectonics of the intestinal muscles of Bayliss and Starling's graphic record (Journal of Physiology, 1899, vol. 24, p. 107). They state that there is synchronous activity at the same spot and at the same time of the two coats (fig. 4, Journal of Physiology, 1899, vol. 24, p. 105). Their record was produced by two enterographs placed at right angles to each other. One records the shortening between *c* and *d*. This represents contraction of the inner coat. The other records shortening between *a* and *b*. This represents contraction of the outer coat. The arrows are directed caudad to the right of the observer.

Fig. 9 This figure represents the intestinal musculature in tonic equilibrium.

Fig. 10 This figure represents a peristaltic wave produced by a stimulus. The stimulus may be a pinch, induction current, or a balloon inserted into the lumen as used by Bayliss and Starling. The inhibition of the contraction in the inner coat is due to the elongation caused by the contraction of the outer coat. The so-called inhibition is found in the zone of caudal dilation. Their records prove that the outer coat causes a widening and continues contracting. Each contraction of the outer coat corresponds to the rhythmic contraction of the cephalic constriction as it progresses caudad.

Fig. 11 The cephalic constriction as schematized has passed the upper point *a*.

Fig. 12 This figure represents the cephalic constriction at the points *c* and *d*. There is now produced a record of contraction of the inner coat. At the same time a record is produced of contraction of the outer coat. This is due to the point *a* approximating *b*. The record shows, therefore, simultaneous contractions, but this does not prove that the effects of the contractions occur at simultaneous points. The resultant of contraction of the outer coat is distal to that of the inner coat in the same unit of time. This is inevitable from the arrangement of the muscle fibers and objectively evident by direct and experimental observations.

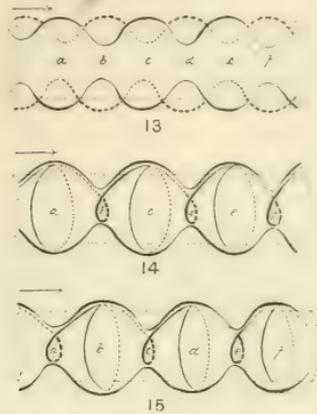
contractions, but the resultant of contraction of the inner coat is a localized cephalic constriction, whereas, in the same unit of time the resultant of contraction of the outer coat is a caudal or distal dilatation. These resultants Bayliss and Starling failed to take into account.

At each rhythmic contraction of the inner close spiral musculature, a simultaneous contraction takes place in the outer muscle layer. The wave of contraction in the outer coat will continue to travel down the intestine. With the next successive and progressive contraction of the inner muscle a second simultaneous wave of contraction is sent through the outer layer. A third, a fourth wave, and so on, are successively started, but the consecutive intervals between the advancing contractions of the outer coat are foreshortened. This leads to a summation of contractions resulting in the caudal dilatation.

This zone of caudal dilatation is produced by manifold contraction waves of the outer coat. Each wave of contraction is immediately preceded by a zone of stretching or tension. Into these elongated areas the trailing contractions take place. The stretching or elongation of the outer is produced by contraction of the inner coat. The latter causes elongation of the intestine and at the same time stretching of the outer muscle coat.

The duplex contraction phenomenon, of a cephalic constriction and caudal dilatation, is due to the structural arrangement of the outer and inner muscular coats of the intestine. A stimulus will start a contraction wave in each simultaneously. The contraction wave traveling in the inner coat in a rotary manner will inevitably trail that of the outer coat pursuing a translatory course. This is comparable to an electric current traveling in a straight wire from *a* to *b* and the same intensity of current traveling in a spirally coiled wire through the same linear distance. It is at once evident that it would take longer for the latter current to reach the same destination on account of the greater distance traveled in a rotary direction as compared to the shorter linear distance traversed by the former.

This analogy may be directly applied to the two contraction waves involved in any type of muscular movement of the in-



Figs. 13 to 15 These figures are diagrams of successive waves of rhythmic contraction. They illustrate the reciprocal elongation of the muscle coats in contraction. Rhythmic contraction is based upon the arrangement of the muscular fasciculi.

Fig. 13 This figure depicts in a reconstruction the alternate contractions and dilatations of two successive rhythmic waves. At the point *a* there is a dilatation simultaneously with contraction at the point *b*. This wave continues on in like manner through the points *c* and *d*, and through the points *e* and *f*. The successive wave is shown by the broken curved lines.

Fig. 14 This figure represents the isolated first wave extending progressively caudad, as depicted in figure 13 as continuous curved lines.

Fig. 15 This figure represents the second or successive isolated wave shown in broken curved lines in figure 13. It is evident that the contraction at the point *a*, figure 15, occurs in the location previously stretched or dilated in figure 14, point *a*. This type of segmentation is produced by successive contraction waves extending through the gut. When the outer muscle coat contracts there is a thickening of the fibers on the proximal side (cephalic aspect) of the area of dilatation. At the same time there is a stretching or elongation of the fibers on the distal side (caudal aspect) of the area of dilatation. This is represented by a thickening and attenuation of the outer lines, respectively. When the outer muscle coat contracts, the resultant shortening and dilatation also elongate the inner muscle coat. Thus, the deformation produced is due to the upsetting of the equilibrium of the two muscle layers by contraction of the outer coat. This stretching stimulus applied to the inner coat results in contraction. The contraction wave progresses into the next stretched zone, and so on down the gut. Rhythmic contraction and diastalsis, therefore, are actions based on the definitive structural plan of the musculature. The effective manifestation of either one or the other of these two actions depends upon the efficient reciprocal elongation of the two sets of intestinal muscles. This is experimentally proved by dissecting off either one or the other of the two layers. An effective block to the propagation of the normal waves is at once established.

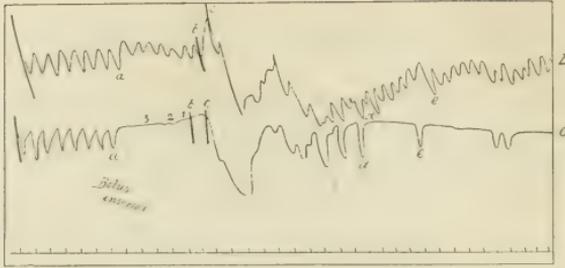


Fig. 16 This figure is taken from Bayliss and Starling's ('99) (*Journal of Physiology*, vol. 24, p. 107, fig. 5) enterographic record of the simultaneous contractions of the outer and inner muscle coats of the intestine. Their interpretation of the record is as follows: "At the beginning of the observation the intestinal wall was contracting rhythmically, the contractions affecting both coats, and being synchronous in both. At (A) a bolus made of cotton-wool coated with vaseline was inserted by an opening into the intestine $4\frac{1}{2}$ inches above the enterographs. It will be seen that the contractions of the circular coat cease instantly and this inhibition is accompanied by a gradually increasing relaxation. There is some relaxation of the longitudinal coat, but the rhythmic contractions do not altogether cease. On inspecting the intestine it was seen that the introduction of the bolus caused the appearance of a strong constriction above it. This constriction passed downwards, driving the bolus in front of it. The numbers above the tracing of the circular fibers indicate the distance of the bolus in inches from the uppermost enterograph lever. At (B) the bolus had arrived at the upper longitudinal lever and at (C) had passed this and was directly under the transverse enterograph, or a little below it. At this point a strong tonic contraction of both coats occurs, expelling the bolus beyond the levers. This strong contraction passes off to be succeeded by another, which like the first is moving down the intestine. In this second tonic wave the rhythmic contractions are evident, superposed on the curve. After the passage of the bolus there is a shortening of the gut (increased tone of longitudinal fibers)."

According to the interpretation of the writer, the 'inhibition' or 'gradually increasing relaxation' seen in the record from *a* to *c*, upon insertion of the bolus above, represents a gradually increasing tension or stretching of both layers as the cephalic constriction nears the contraction record beginning at *C*. Immediately preceding each contraction of the outer layer there is a zone of tension into which each wave of contraction enters. The caudal dilatation, therefore, is a summation of advancing contraction waves closely following one another. This is objectively evident in the curve for the outer layer, *L*, from *a* to *c*, in which rhythmic contractions are taking place. Immediately preceding each contraction is a zone of stretched muscle fibers, the sum of which would tend to lengthen slightly the caudal dilatation over that of the same length of intestinal musculature either in tonic equilibrium or in rhythmic segmentation. The more rapid rhythmic contractions and increased stretching of the outer layer throw the balance of equilibrium between the outer and inner layers in favor of the former. The inner layer in the area of caudal dilatation is held, therefore, in a state of gradually increasing tension or stretching until the optimum at the point *e* is reached. At the point *c* both layers record contraction waves, but it must be remembered

testine. The contraction wave traveling in a rotary manner in the inner close spiral muscle will trail the contraction wave traveling in the outer elongated spiral muscle. The former and stronger contraction will cause a cephalic constriction, the latter and weaker contraction will cause a caudal dilatation (figs. 7 and 8).

The fasciculi of the inner coat make one complete turn in every 0.5 to 1 mm., whereas those of the outer coat make one complete turn in every 200 to 500 mm. Consequently, if we consider the fasciculi as not interconnecting, which they do, however, we will find that a contraction wave that travels 5 mm. in a linear direction through the inner coiled muscle coat will find its complimentary contraction wave that started at the same time and place in the outer coat to have traveled approximately 300 mm. in a translatory direction. This estimate is based on an intestine 25 mm. in diameter (figs. 18, 19, 20, 21). The intestinal movements are rhythmic, however, and this characteristic is due to waves transmitted in each coat at each duplex contraction, thereby causing a reciprocal elongation and mutual coördination of the two waves characterizing peristalsis or segmentation (figs. 13, 14, 15).

that these contraction waves at any period of observation occupy areas of former tension. The tension of the inner coat is caused by the outer one and the tension of the outer coat is caused by the inner one. The double phenomenon of tension prior to contraction travels quickly down the intestine in the outer coat. The relationship of prior tension and trailing contraction is comparable to a gymnast climbing a rope. The tensed rope in advance corresponds to the zone of tension in the intestinal musculature and the progressing gymnast represents the advancing contraction wave.

The graphic record also strikingly shows that the waves travel in a translatory direction much faster in the outer than in the inner muscle layer due to the rotary direction of the path in the latter. The record shows that seven waves in the outer coat have passed the recording point before the inner wave of contraction has reached the point *b*. This fact is also verified after the main rhythmic tonic waves of diastalsis have passed the recording levers at *d* and *e* (*d* and *e* were inserted by the writer). The intestinal muscles are now tending to equilibrium and have not reached as yet the state in which each contraction of the outer and inner coats show the simultaneous contraction curves prior to the insertion of the bolus. *L*, contraction curve of outer fibers. *C*, contraction curve of inner fibers. Contractions recorded by down strokes of enterographic lever, relaxations by upward movement of lever.

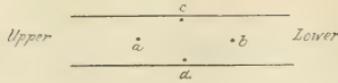
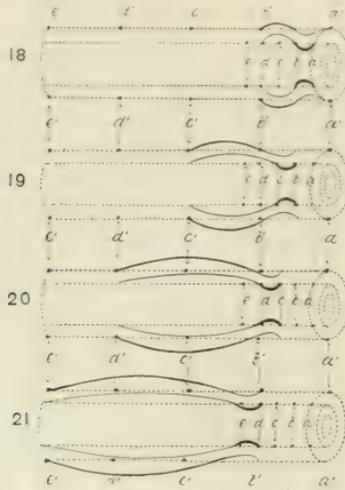


Fig. 17 This figure represents the position of the enterographs which were placed at right angles to one another in Bayliss and Starling's experiment (*Journ. Physiol.*, vol. 24, p. 107, fig. 5a) *a* and *b* are the levers of the enterograph recording the contractions of the inner muscle coat.



Figs. 18 to 21 These figures represent the initial progressively increasing caudal dilatation synchronous with successive contractions of the inner coat cephalad.

Fig. 18 This figure shows contraction of the inner coat from *a* to *b* simultaneous with the contraction of the outer coat through one-half the distance from *a'* to *b'*. The contraction wave of the outer and inner coats are preceded by zones of tension or stretching of the muscle fibers produced by the reciprocal elongating actions of the two muscle layers.

Fig. 19 This figure represents the contractive wave of the inner coat advancing from *b* to *c* synchronous with an increasing caudal dilatation produced by the outer coat midway between *a'*, *b'* to *c'*.

Fig. 20 This figure depicts the caudal dilatation still further advanced from *b'* to *d'* at the same time that the inner constriction travels from *c* to *d*.

Fig. 21 This figure demonstrates the advancing contraction wave of the inner coat occupying the distance from *d* to *e*. The caudal dilatation extends from a point midway between *b'*, *c'* to *e'*. With each successive rhythmic constriction produced by the cephalic contraction wave in the inner muscle fibers a corresponding wave is initiated in the outer muscle layer. The summation of the contraction waves in the outer group of fibers produces the caudal dilatation due to the greater activity of these fibers over that in the inner layer. The balance or equilibrium is upset, therefore, in favor of the outer fibers at this point.

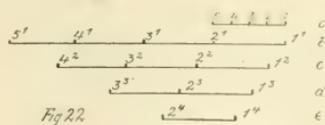


Fig. 22 This figure is a diagram representing the differential rates of translatory advance of the successive contraction waves in the outer and inner layers of muscle fibers. Line *a* represents the inner close spiral muscle fibers. Lines *b*, *c*, *d*, and *e*, the outer elongated spiral muscle layer. Upon stimulation there is produced a rapid caudal dilatation and a slowly produced cephalic constriction. Contraction waves begin simultaneously, in both coats, but, due to the linear course followed by that in the outer muscle layer, a difference in time takes place before the effects of contraction are objectively evident in the two layers of muscles. When the cephalic constriction advances from 1 to 2 line *a*, the caudal dilatation is evident from 1' to 2' line *b*. When the cephalic constriction progresses still further from 2 to 3 line *a*, the caudal dilatation is a summation of two contraction waves in the outer fibers from 2' to 3' line *b*, and 1'' to 2'' line *c*. Likewise with continuation of the constriction produced by the inner coat from 4 to 5 line *a*, the caudal dilatation is a summation of four consecutive waves closely following one another, namely, 4' to 5' line *b*, 3'' to 4'' line *c*, 2''' to 3''' line *d*, and 1'''' to 2'''' line *e*. From this it is evident that the degree of caudal dilatation is directly proportional to the extent of the cephalic constriction. If the cephalic constriction is a long trough of 5 cm. or more, the summation of caudal constrictions in the outer fibers is greater than if only 1 cm. is involved in the formation of the cephalic constriction. This explains the difference of extent of the caudal dilatation manifested in the intestinal movement of segmentation and diastalsis.

Conclusions

The Screw-like Action of the Small Intestine

1. Because of the left-handed, helicoidal arrangement of the musculature of the intestine, the intestinal movements are comparable to the action of a left-handed screw.

2. The fasciculi of the inner coat make one complete turn in every 0.5 to 1 mm., whereas those of the outer coat make one complete turn in every 200 to 500 mm. Consequently, if we consider the fasciculi as not interconnecting, which they do, however, we will find that a contraction wave that travels 5 mm. in a linear direction through the inner coiled muscle coat will find its complimentary contraction wave that started at the same time and place in the outer coat to have traveled ap-

proximately 300 mm. in a translatory direction. This estimate is based on an intestine 25 mm. in diameter.

3. *The inner muscular layer is wound as a close spiral. The outer as an open spiral. The difference in rate of translatory progression of the two contraction waves depends upon this muscular arrangement. The wave traveling in the inner group of fibers takes a rotary course, whereas that in the outer fibers takes a more translatory course to reach a corresponding destination. Therefore, the contraction of the stronger inner muscle coat will inevitably trail that of the outer. The arrangement of the intestinal muscle layers clearly explains the phenomenon of cephalic constriction and caudal dilatation of diastalsis without invoking the aid of hypothetical nerve paths. Peristalsis, therefore, is a duplex contraction phenomenon produced by the differential rate of translatory advance of the two contraction waves in the outer and inner muscle layers, respectively.*

4. By exsection of the inner muscular layer of the intestine, it is proved that the caudal dilatation of diastalsis is produced primarily by the summation of contractions in the outer layer of muscle fibers.

5. By exsection of the outer muscular layer, it is proved that the cephalic constriction is caused by the inner layer of close-spiral fibers.

6. The movements of the intestine depend upon the reciprocal elongating actions of the outer and inner layers of muscle fibers, respectively. These movements are superimposed on the tonic condition of equilibrium of the two muscle layers. The application of any drug, therefore, that decreases the excitability of the musculature or destroys the tonic equilibrium of the two layers will indirectly effect the typical muscular responses.

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Resumen por el autor, Naohide Yatsu.

Sobre los cambios de los órganos reproductores en la parabiosis heterosexual de ratas albinas.

En las parabiosis de macho y hembra (individuos unidos mediante una operación quirúrgica), algunos de los folículos de Graaf se desarrollan normalmente y forman cuerpos amarillos, mientras que la mayoría experimenta cambios regresivos. En estas parabiosis el útero no se modifica de modo marcado, aun cuando puede presentarse una hiperplasia de la subserosa. Los folículos ováricos de las parabiosis de macho y hembra castrado no se desarrollan normalmente, ni tampoco se forman cuerpos amarillos. Los quistes folículos y los cuerpos atréticos son abundantes. Hay un aumento aparente de las células intersticiales, entre las cuales existen unas cuantas células luteínicas. El útero es el órgano más afectado mediante la unión con un macho castrado. Especialmente clara es la producción de hidrometrias. El testículo y la próstata no son afectados por la unión con hembras normales o estériles.

Translation by José F. Nonidez
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ON THE CHANGES IN THE REPRODUCTIVE ORGANS IN HETEROSEXUAL PARABIOSIS OF ALBINO RATS¹

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SEVEN FIGURES

The actions of products from various endocrine organs have been studied of late so extensively that one can hardly keep in touch with the literature relating to even a single organ. Of the methods employed for such investigations parabiosis has not so far been used as it ought to be. And, I think, much new light will be thrown in the future upon endocrinology by making use of this method.

In the fall of 1916, several series of experiments in joining together two albino rats of different sexes were begun at the Zoological Institute of the Imperial University, with the help of Mr. Masanosuke Takesita.² The object of these experiments was to obtain evidence regarding the effects of male and female hormones in mixed condition upon united individuals. The present investigation was more immediately suggested by Lillie's paper on the theory of the free-martin which appeared in "Science" ('16). In this paper he expresses the view that in the heterosexual twins of cattle the reproductive organs of the female fetus are impaired by the influence of sexual hormones from the male fetus, provided a blood circulation has been established

¹ In carrying out the experiments described in this paper, I am indebted to the Tokugawa Memorial Fund.

² Mr. Takesita's death took place rather suddenly in November of 1919, just after the operative part of the present investigation came to an end. He graduated from the Imperial University in 1916. At the time of his death he was thirty years old. I owe a great deal to his skill and intelligence in carrying out this study.

between the two. I thought it would be interesting to see how the male hormones act upon the female and vice versa in adult heterosexual parabiosis.

As to the method of parabiotic operation I am indebted not a little to Dr. Rikurô Matsuyama. His kindly advice was invaluable. When I began this work he was engaged in a study of uraemia produced by extirpating the kidneys from one component of rat parabioses. Later, however, he directed his attention to the line which I was following. He obtained results almost the same as mine, and published an excellent paper in Japanese in August, 1919. Yet, since his paper is written in a language only slightly intelligible to our confrères outside this country and because of the existence of discrepancies in our work and of additional observations, the publication of the present paper may not be regarded as superfluous.

Most of the rats used for the experiments were bred in the laboratory. Their ages, body length and body weight were noted at the time of operation (parabiosis and gonadectomy) and when killed. All the parabiotic pairs from which the material was taken had been healthy. It is, therefore, almost certain that any alteration found in the preparations is due to the action of mixed hormones produced by united individuals.

MALE-FEMALE PARABIOSES

I have twenty-four successful cases of male-female parabioses. The period of union ranges from 11 to 179 days. It need hardly be mentioned that the actual parabiotic period does not correspond with that of union for the blood circulation becomes established between two animals only after at least ten days. The youngest animal operated on was twenty-nine days old and the oldest ninety days old. The individuals used for parabiosis were in some cases from the same litter and in others from different litters, but in all cases they were of the same age.

1. Changes in the ovary

The ovaries of parabiotic pairs were cut into sections and compared with those of unoperated females at corresponding ages, which had been kept separated from the males.

At the outset it might be mentioned that the time of appearance of the first corpus luteum, that is, the first ovulation comes at about the same time in the ordinary females and in the parabiotic females. Ovulation may continue for a considerable length of time, as is indicated in a case (161-day-old female, 111 days in parabiotic union) in which several apparently normal eggs at the metaphase of the second maturation mitosis were found in the oviduct. And this is also substantiated by the fact that both Morpurgo ('08) and Matsuyama '19) obtained normally developed fetuses from parabiotic females.

While the formation of the corpora lutea is taking place on the one hand, a great many graafian follicles are found undergoing regressive changes on the other. In fact, many more follicles degenerate in the ovaries of parabiotic females than in ordinary females. This I do not hesitate in attributing to the influence of the male component. Predominance of degenerating follicles makes itself evident after thirty days' union with the male. The longer the parabiotic period, the larger the number of degenerating follicles. It may also be added that the regressive changes set in more in younger follicles than older ones.

There are several modes of degeneration of the follicles. But it is important to note that none of the modes is peculiar to parabiosis. The only difference found between those in the normal ovary and in that of parabioses is in degree of change.

The first indication of degeneration of the follicle is the dissolution of the cells composing the cumulus oöphorus. First the egg is set free in the follicular cavity. Then it is divided or rather fragmented into five or six cells of different sizes in the manner described by Hennuguy ('94) in the rat and by Spuler ('01) in the guinea-pig. Sometimes there are two nuclei in one cell. These 'blastomeres' disintegrate in the follicular fluid so completely that no trace of them can be found. The above changes also take place in very young follicles.

Pari passu with the dissolution of the egg goes the disintegration of the stratum granulosum. This is accomplished in two ways. The first mode is the gradual dissolution of the component cells from the inside, reducing the layer to a very thin sheet of cells, often separated from the theca interna by a narrow space. In an advanced stage, this layer is fenestrated, appearing in sections as an interrupted ring. The other means of destruction is by phagocytes that have entered the follicle from without. Whence they come I am not able to ascertain. At any rate, the phagocytes are much larger than the granulosum cells and



Fig. 1 Portion of a degenerating follicle (forty-seven days of parabiotic union). *P.*, phagocytes; *G.-st.*, granulosum; *I.*, theca interna; *E.*, theca externa. $\times 560$.

are readily detected by ingested débris. They migrate toward the follicular cavity until they occupy a position in the fluid, as is shown in figure 1. As to the fate of these cells, I am unable to offer any suggestion. It is very probable that they also disintegrate in the follicular fluid.

As the granulosum layer disappears, the theca interna increases in thickness. Sometimes this process goes on only in a restricted area, sometimes along the entire layer. In either case the cells constituting the theca interna increase in size. These cells however, are quite different from the lutein cells. The nucleus and the cell body of the former are much smaller, more granular, and stain more deeply with haematoxylin than those of the latter.

The cytoplasm of the lutein cells is more eosinophilic. In cases where the central cavity is obliterated, a corpus atreticum composed of modified theca interna cells is formed.

Side by side with the above-described atretic follicles are found those with the theca cells modified into the lutein cells. Such follicles have often a large central cavity. Matsuyama thinks that this type is peculiar to the ovaries of parabioses, but in my sections of the ovaries of old females this kind of follicle is not uncommon.

A feature that may rightly be called characteristic of the ovaries of parabiosis is the enormous growth of the interstitial cells.

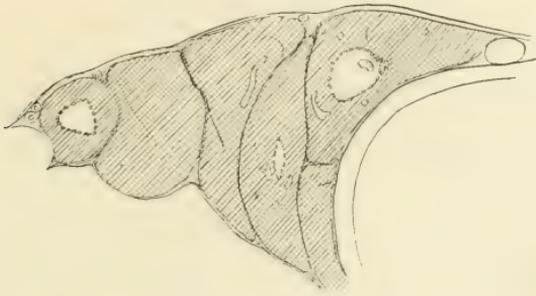


Fig. 2 Portion of ovary showing six groups of interstitial cells (forty-seven days of parabiotic union). $\times 86$.

Matsuyama has noticed this peculiar change also. This is met with especially in the female, which is operated upon when young; that is, at a stage in which the follicles have not yet attained their full size.

It cannot be doubted that the interstitial cells are modified theca cells. Consequently, the follicular cavity is found, in most cases, inside the mass of these cells and in some cases degenerating ova and débris of the granulosa layer, as is shown in figure 2.

The lutein cells are sometimes found among the interstitial cells. They can readily be distinguished, since the former have large nuclei and a single nucleolus in each, while the latter

have small nuclei, with scattered chromatin granules. Is the formation of lutein cells due to immigration or transformation? I think both processes may go on, judging from the fact that few mitotic figures are met with.

In conclusion it may be said that in parabiosis the male exerts a certain deteriorating effect upon a good many graafian follicles of various stages, though some of the follicles remain apparently normal and are able to discharge fecundable eggs. The injurious effect of the male hormones does not produce anything peculiar to parabiosis, but accelerates degeneration processes that would take place in normal ovaries

2. Changes in the uterus

The changes in the uterus of the parabiotic female are usually not so marked as those in the ovary. As a matter of fact, changes, if any, are so slight that they do not at all affect the normal development of the fetuses, as was shown by the cases of Morpurgo and Matsuyama.

In some females, however, changes are noticeable after a month of parabiotic union. The hyperplasia of the stratum subserosum is the main feature. The uterine glands decrease in number. The muscular layers become thinner. The eosinophilic leucocytes seem to be more abundant than in the normal uterus.

It is interesting to note that in two cases the uteri have been modified exactly like those of female+castrated male parabioses, which will be described in the next section. In the two cases the testes were smaller than in other males of parabiotic union, though spermatogenesis was going on normally.³

³ In one of the two males (139 days old when killed, 93 days of parabiotic union, body weight 160 grams, body length 182 mm.) the right testis was 0.977 gram and the left 0.967. In the other male (159 days old when killed, 106 days of parabiotic union, body weight 195 grams, body length 170 mm.) the right testis was 0.338 gram and the left 0.310 gram. In comparison it may be stated that in the males of similar age and of similar length of parabiotic period the testes weigh between 1.100 and 1.200 grams.

FEMALE + CASTRATED MALE PARABIOSES⁴

Males were castrated, and after various intervals they were united with females. I have fourteen successful cases of this kind of parabioses. The period of union ranges from 18 to 179 days.

Contrary to what one might expect, the changes in the ovary and the uterus are more marked in these cases than in the above-described female + uncastrated male parabioses.

1. Changes in the ovary

The ovary is so affected by the influence of the castrated male that it ceases to discharge the eggs, judging from the fact that no normal corpora lutea are formed and what look like them are nothing more than the corpora atretica. All the follicles undergo regressive changes of one kind or another. The processes of degeneration are different in follicles of different stages of development. They are the same as those described by Böshagen ('04), Benthin ('11), Cohn ('09), and others. It may here be mentioned that none of the regressive changes is peculiar to this kind of parabiosis, all being met with in the normal ovary, as is the case with male-female parabioses. But all the modifications come in intensified form.

Of several modes of change of the follicles, I may mention first of all a most striking one, which I would not hesitate to regard as characteristic of this type of parabiosis. This is cyst formation due to enormous growth of the stratum granulosum. One example is shown in figure 3. Here one sees uneven growth of this layer. As the follicular fluid accumulates the follicle is distended, reducing the wall to extreme thinness. Sometimes the blood-vessels make their way into the cumulus oöphorus. The egg in the follicular cysts usually undergoes degeneration without fragmenting. It may occasionally divide, but as far as I know it does not act as in younger follicles.

⁴Harms ('11) has described a case of this kind of parabiosis in *Rana temporaria*, but I am not able to learn from his paper how the female organs were affected by the castrated male.

This remarkable growth of the granulosa layer takes place not long after parabiotic operation. Indeed, I have a case in which the ovary showed this change only eighteen days after union.

At one portion of the follicular cyst one often sees an accumulation of the lutein cells formed from the theca interna. I have no strong evidence to oppose the view that this represents a stage on the way to formation of a corpus atreticum. But I am rather inclined to believe that any follicle once distended on the way to become cystic, will remain as such and never transform into the solid corpus atreticum. As a matter of fact lutein cells are found in the wall of half-grown follicles. This is represented in figure 4. Here the lutein cells are formed not only from the

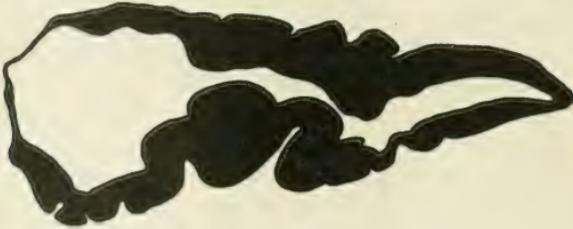


Fig. 3 Stratum granulosa of a follicle (thirty days of parabiotic union).
× 86.

theca interna directly, but also from the interstitial cells. After the disintegration of the granulosa cells of such follicles they turn into solid corpora atretica though sometimes a small cavity appears in them.

Other changes, such as unusual growth of the interstitial cells, are exactly the same as in male-female parabioses.

2. Changes in the uterus

The uterus, of all the female organs I have examined, is the most affected by the union with a castrated male. The striking change makes itself evident as early as eighteen days after parabiotic operation. The normal structure of the uterus will not here be described, since that has been so fully studied by Po-

wierza ('12) in the mouse and by Beiling ('06) in the rat. The first indication of the change is the rapid growth of the mucous layer. This is soon followed by the accumulation of turbid fluid in the uterine cavity. The subserosa or vascular layer, with not a few eosinophils, becomes thinner. The uterine glands may remain for some time compressed within the now narrow

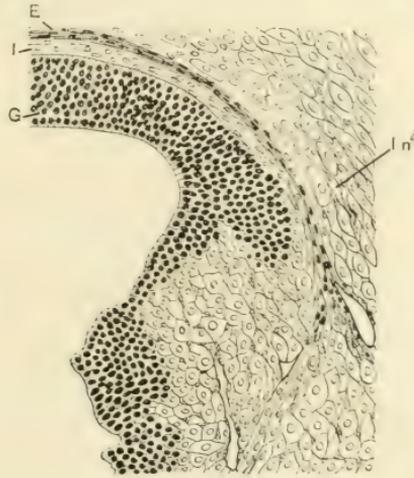


Fig. 4 Part of a follicle showing invasion of lutein cells into st. granulosum (*G.*) from interstitial cells (*In.*) (107 days of parabiotic union). *I.*, theca interna; *E.*, theca externa. $\times 186$.

subserosa. Finally they disappear completely. The longitudinal muscles are no longer found. The diameter of this distended uterus is 6 mm., while that of the normal one is not more than $1\frac{1}{2}$ mm. The wall is reduced to one-tenth the normal thickness. This hydrometral condition is not accompanied by hydrosalpinx, which Fischel obtained experimentally ('14). No noticeable changes take place in the tubal part.

TESTES OF PARABIOSES

The testes of male-female parabioses (twenty-four cases from 11 to 179 days) and of male+spayed female parabioses (eleven cases from 6 to 148 days) were weighed and cut into sections.

It is interesting to note that the histologic structure of the testes is not affected at all by the union with either unoperated

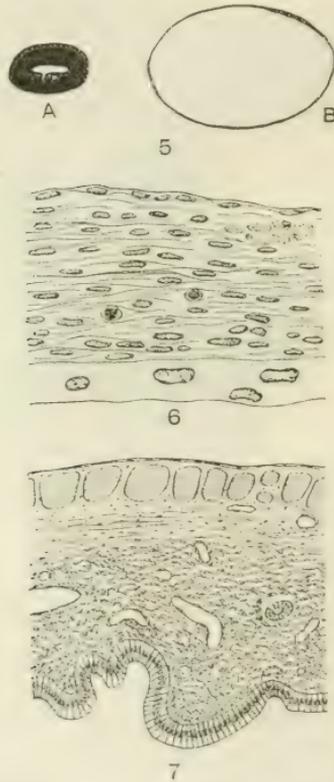


Fig. 5 Section of normal (A) and distended uterus (B). $\times 4.5$.

Fig. 6 Portion of distended uterus (eighteen days of parabiotic union). Notice the remnant of longitudinal muscle fibers. $\times 420$.

Fig. 7 Portion of half-distended uterus (twenty-three days of parabiotic union). Here one notices a uterine gland to the right. $\times 97$.

or spayed female. In the latter combination, however, the sperm formation is somewhat delayed. In one case the spermatozoa could not be seen in a ninety-eight day rat joined for thirty-two days with a spayed female.

RESULTS

That the spermatozoa are functional in male-female parabioses is shown by the fact that a female which had been kept with a male-female parabioses gave birth to a litter. Copulation took place in this case 134 to 135 days after parabiotic operation.

In passing it may be mentioned that the prostate is not affected at all by the union with either normal or spayed female. In castrated male-female parabioses the prostate atrophies as in the solitary castrated male.

1. In male-female parabioses some graafian follicles undergo the normal course of growth and the corpora lutea are formed, while a large majority of follicles undergo regressive changes. None of the changes is peculiar to this kind of parabiosis.

2. In male-female parabioses the uterus is not modified very markedly. Sometimes hyperplasia of the subserosa is noticed.

3. In the ovary of castrated male-female parabioses none of the follicles develops normally. No corpora lutea are formed. Follicular cysts and corpora atretica are abundantly produced. Noticeable growth of the interstitial cells takes place and sometimes the lutein cells are met with in the interstitial cell groups.

4. The uterus is most affected by the union with a castrated male. Hydrometra of various grades is the remarkable feature. The uterine tubes are normal.

5. The testis is not affected at all by the union with either normal or spayed females. The same is true of the prostate.

COMMENT

From the above-described parabiotic experiments rather unexpected results were obtained. One would naturally suppose that the female organs would be more affected by the male with the testes. But as a matter of fact they are more influenced

by the castrated male. To account for this phenomenon I think it is very probable that the endocrine organs of the male are affected by castration, and that the ovary and uterus of united individuals are in turn influenced by the hormone or hormones produced from these organs. But what organ partakes in this process and how I do not know.

It is also interesting to note that the testis is not impaired in the least by the ovary of the female to which it is united.

Anatomical Institute, Keio University,
July 22, 1920

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2001

Resumen por el autor, Howard Homer Bell.

Divertículos del duodeno. Descripción de tres observaciones.

El primer caso estudiado presentaba un divertículo en la región de la ampolla de Vater. El segundo presentaba un divertículo en la región de la ampolla mencionada y otro más inferior en la segunda parte del duodeno; ambos pacientes eran varones y marcadamente obesos. Su edad era cuarenta y dos, y sesenta y un años, respectivamente. El tercer caso presentaba tres divertículos, uno en la papila mayor, otro en la menor y un tercero en la unión de la segunda y tercera parte del duodeno. El paciente era una hembra emaciada de setenta y cinco años de edad. Divertículos semejantes se presentan también en el colon. En todos los casos estudiados la muscularis terminaba bruscamente sin formar parte del saco. Estos divertículos se consideran como adquiridos a consecuencia de la existencia de puntos débiles en la muscularis.

Translation by José F. Nonidez
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DIVERTICULA OF THE DUODENUM

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TWO FIGURES

Diverticula have been observed in all divisions of the gastrointestinal tract. Particular attention, however, is given to diverticula of the duodenum on account of their relation to the common bile and pancreatic ducts and associated organs.

Diverticula of the alimentary tract occur most frequently in the colon, ileum, oesophagus, pharynx, duodenum, and stomach,—in the order named (Buschi).¹ Baldwin² observed fifteen cases of duodenal diverticulum in 105 cadavers. Schüppel (quoted by Baldwin) found seven instances in forty-five bodies, and later at Kiel found one in 200 bodies. Linsmayer³ observed forty-five cases in a study of 1367 autopsies.

Davis⁴ found from a study of the literature that 16 per cent of duodenal diverticula occurred in subjects under fifty years, 37 per cent between fifty and sixty years, and 47 per cent above sixty years. In the series studied by Linsmayer, the youngest showing diverticulum was thirty-six years and another thirty-eight years old. Three instances occurred between forty and forty-nine years, 5 between fifty and fifty-nine years, 13 between sixty and sixty-nine years, 14 between seventy and seventy-nine years and 4 between eighty and eighty-seven years. Wilkie⁵

¹ Virchow's Arch. f. path. Anat., 1911 (206), 121.

² Anat. Rec., vol. 5, 1911, p. 121.

³ Verhandl. d. deutsch. path. gesellsch. 17, 445, 1914.

⁴ Tr. Chicago Path. Soc., vol. 9, 1913, p. 1.

⁵ Edinburgh Med. Jour., vol. 11, 1913, p. 219.

found that in twenty-six cases, seventeen were in males and nine were in females. He believes that diverticula of the duodenum are congenital in origin and bases this belief upon the following data:

1. "The duodenum in the course of normal development gives off hepatic and pancreatic buds; consequently developmental anomalies might be expected to occur more frequently in this than in other regions of the intestinal tract. Tandler ('02) found that congenital atresia occurs in the duodenum 39.6 times as frequently as in any other segment of small intestine of equal length.

2. "The observation of Lewis and Thyng ('08) showed that during early foetal life, diverticula are often met with in the the upper reaches of the small intestines, particularly in the duodenum.

3. "An accessory pancreas is occasionally observed in or on the wall of such diverticulum.

4. "The case reported by Shaw, where in a newborn infant there were found both a diverticulum and a congenital occlusion of duodenum.

5. "In the cases recorded by Letulle ('98) and Falconer ('07) congenital diverticula of the oesophagus and stomach, respectively, accompanied the duodenal diverticula.

6. "Many of these diverticula abut on and even indent the head of the pancreas."

More than 400 diverticula were observed in the gastro-intestinal tract of an adult male (Hansemann⁶). These were situated along the line of attachment of the mesentery corresponding to the points of penetration of the larger vessels, particularly the veins. This relationship of vessels to diverticula has not infrequently been observed. Relaxation of venous sheaths in association with circulatory stasis (Graser⁷), fatty degeneration of the muscularis, (Roth⁸), and fatty infiltration of the intestinal

⁶ Virchow's Arch. f. path. Anat., 1896, Bd. 144, S. 400.

⁷ München. med. Wehnschr., No. 22, 1899, S. 721.

⁸ Virchow's Arch. f. path. Anat., 1872, Bd. 56, S. 197.

wall (Aschoff⁹) were conspicuous in certain cases and considered as the causes of the diverticula.

Baldwin found from the literature that the muscularis was observed in the walls of these diverticula in twenty instances, while in sixteen it was absent. In forty-seven cases there was no report on this subject. (Keith¹⁰) states that it is not uncommon to find in old people in the posterior wall of the duodenum near the termination of the common bile duct diverticula which are pouches of mucous membrane extruded at weak points in the musculature. However, he cites the cases described by Clogg and Thompson as instances of developmental diverticula; these occurred in the small intestine below the duodenum in association with accessory pancreas, and possessed the muscular coats of the intestine.

The arrangement of the muscularis is subject to great variation and aids very little in a study of the etiology of these diverticula. Meckel's diverticulum possesses the musculature of the intestinal tract. Its origin is peculiar and suggests no explanation of diverticula elsewhere.

In most instances diverticula of the duodenum were found at necropsy and had no direct relation with the cause of death. However, in a case reported by Bauer,¹¹ biliary stasis with jaundice was caused by inflammation of a diverticulum at the site of the ampulla of Vater. Death followed intrathoracic hemorrhage. Furthermore, several instances of duodenal diverticula occurred in association with pain in the upper abdomen and disturbances of digestion; either duodenal ulcer or gall-bladder disease was found in this group of cases, which possibly accounted for the symptoms present. However, disturbance of digestion, poor nutrition, and certain nervous manifestations have occurred in association with duodenal diverticula, which were found by x-ray examination or at operation, symptoms that could not be

⁹ Pathologische Anatomie, dritte Auflage, zweites Band, S. 824: Jena, Gustav Fischer, 1903.

¹⁰ Brit. Med. Jour., 1910, p. 376.

¹¹ Wien, klin. Wehnschr., 1912, (25) 879.

explained otherwise than by the diverticula. A diverticulum has led to surgical intervention or has been found at operation and corrected in several instances. Consideration of the facts presented in the literature indicates that the significance of the associated symptoms is uncertain.

I have examined two specimens of diverticulum of the duodenum. The first instance occurred in a teamster, forty-two years old, who related no symptoms indicating that this diverticulum had given him any trouble. He was very obese. Death followed failure of cardiac compensation associated with aortic stenosis and chronic fibrous myocarditis. The anatomical diagnosis made at necropsy was as follows:

Aortic stenosis and insufficiency; hypertrophy and dilatation of heart; recent and old infarcts of lung; chronic passive congestion of all organs; chronic tuberculous process of lung and spleen; nephrolithiasis; obesity; diverticulum of duodenum.

The diverticulum occurred as a blind pocket immediately above and anterior to the major papilla. It communicated with the duodenum through a puckered opening measuring 0.5 cm. in diameter. It was about 2 cm. deep and 1 cm. wide. It extended to the left and slightly upward anterior to the common bile and pancreatic ducts in the fissure between the lobes of the head of the pancreas. The common bile duct was slightly dilated and opened independently into the duodenum beside the pancreatic duct, being separated from it by a very narrow septum. The sac was formed by thinned intestinal wall. No vessels were found which penetrated the sac. The situation of this diverticulum is shown in figure 1.

Sections were made from the wall of the diverticulum. The muscularis ended fairly abruptly and did not form a part of the sac. Considerable fat was deposited in the intestinal wall and some occurred in the muscularis.

The second instance occurred in a man sixty-one years old and showed two diverticula. No symptoms referable to the diverticula were recorded. The patient had been employed in an organ factory. He was very obese and suffered from a long-standing irreducible inguinal hernia. The hernia was

reduced and repaired by operation. The caecum, which was greatly dilated and distended, filled the sac. The patient died a few days following the operation, from peritonitis associated with many ulcers and numerous perforations of the caecum, ascending and transverse colon. The anatomical diagnosis made at necropsy was as follows:

Operative incisions in right thigh and inguinal regions; repair of hernia with fascia transplantation; peritonitis following intestinal ulceration and perforation; follicular enteritis; chronic fibrous myocarditis; arterial sclerosis; focal sclerosis of endo-

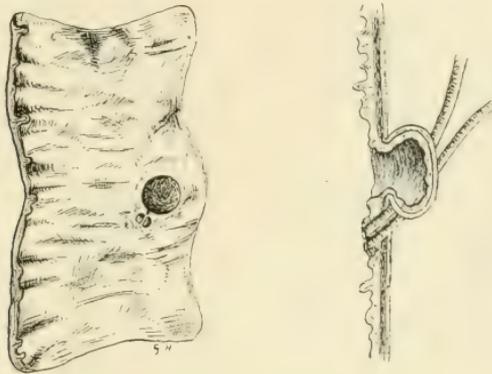


Fig. 1 Drawing showing the inside and lateral views of the duodenum and the relation of the diverticulum to the openings of the common bile and pancreatic ducts.

cardium, aortic and mitral valves; chronic diffuse nephritis with atrophy of cortex and fatty degeneration of pyramids; chronic passive congestion of viscera; chronic bronchitis; fatty degeneration of liver and kidneys; chronic obliterative appendicitis, prostatic hypertrophy; healed tuberculosis of peribronchial lymph nodes; old pleural adhesions; obesity; lipomatosis of pancreas with fat necrosis; diverticula of duodenum; patent foramen oval; accessory spleen.

The first diverticulum occurred at the site of the ampulla of Vater. It communicated with the duodenum through an oval opening measuring about 1 cm. vertically and 0.6 cm. horizontally.

It was about 2 cm. deep and terminated as a blind pocket. The diverticulum extended to the left and slightly upward in front of the common bile and pancreatic ducts in the fissure separating the two lobes of the head of the pancreas. The common bile duct was moderately dilated and opened into the posterior and lower part of the diverticulum, about 0.5 cm. from the intestinal wall, in conjunction with the pancreatic duct. The sac was formed throughout by thinned intestinal wall.

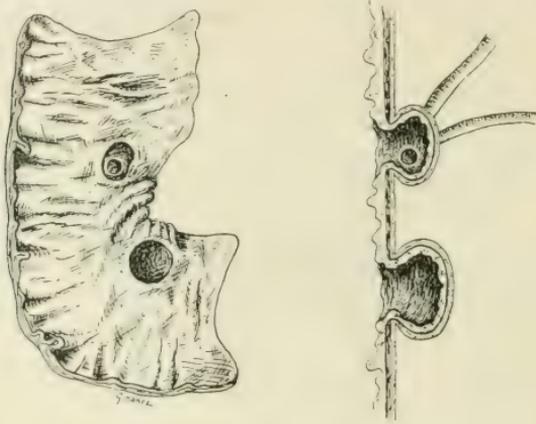


Fig. 2 Drawing showing the inside and lateral views of the duodenum and the relation of the diverticula to the duodenum and the common bile and pancreatic ducts.

The second and larger diverticulum occurred in the lowest part of the duodenum along the posterior surface and rested chiefly upon the vena cava. It was disc shaped, being flattened anteroposteriorly. It communicated with the duodenum through an opening 0.9 cm. in diameter. After fixation the sac measured 3 cm. in its longest dimension and 1.5 cm. in its shortest. It was about 2 cm. deep. The sac was formed by thinned intestinal wall. The anatomical relations of these diverticula are shown in figure 2.

Sections from the walls of the diverticula show that the muscularis ended rather abruptly and did not form a part of the sac.

Considerable fat was deposited in the intestinal wall and some occurred in the muscularis.

The two cases herein reported show some of the conditions frequently observed in association with duodenal diverticula. The patients were male and moderately advanced in life, i.e., forty-two and fifty-one years old, respectively. Each showed much venous stasis. Obesity was marked in both instances. The muscularis ended rather abruptly about the openings and did not form a part of the walls of these diverticula.

A consideration of the facts presented in the literature and of the cases reported here leads one to the conclusion that duodenal diverticula of this type are not congenital, but acquired, occurring at points in the muscularis which are weakened by the passage of ducts and blood-vessels or by pathological processes, and that increasing age and the greater muscular activity in men are factors in their production.

SUPPLEMENTARY REPORT

I have recently studied a specimen, with three diverticula of the duodenum, removed at autopsy.

The patient was a woman seventy-five years old, and was markedly emaciated. No symptoms referable to the diverticula were recorded. There was general arterio-sclerosis with gangrene of the feet. Death followed acute parotitis and micrococcus aureus was grown from the parotid gland and the heart's blood.

The anatomical diagnosis was as follows: General arterio-sclerosis; gangrene of the feet; acute parotitis; volvulus of the caecum and transverse colon; carcinoma of cervix uteri with extension and metastasis; chronic nephritis with small contracted kidney; healed calcified tubercles in the lungs, bronchial lymph nodes and spleen; diverticula of the duodenum and colon; chronic interstitial pancreatitis; periportal cirrhosis of the liver.

The largest diverticulum occurred at the junction of the second with the third part of the duodenum, along the upper border, 22 mm. below the major papilla. It was saccular in shape and extended upward, inward and backward. The opening was

oval, measuring 15 mm. by 8 mm.; the longer axis was paralld with the folds of mucous membrane which at that point extended obliquely downward and outward. The sac measured 18 mm. deep. A small artery and vein crossed the outer wall of the sac and entered the intestinal wall at the base of the pouch.

The second diverticulum occurred immediately to the right of the bile papilla and extended upwards, outward and backward along the right side of the common bile duct. Its opening was a crescentic shaped slit which encircled the outer half of the ampulla. The common bile duct and the pancreatic duct opened independently into the duodenum; there was no ampulla of Vater. The diverticulum was saccular in shape and measured 15 mm. deep. It was empty, but pressure upon the gall bladder filled it with bile. A transverse fold of the mucous membrane hung down over the bile papilla and the opening of the diverticulum. No vessels were observed in the walls of this diverticulum.

The third diverticulum occurred immediately above the minor papilla. It was conical in shape and embedded in pancreatic tissue along the duct. It measured 8 mm. at the opening and 12 mm. deep. It was situated 18 mm. above the major papilla. A transverse fold of mucous membrane hung downward over it.

The muscularis ended abruptly at the opening of these diverticula and did not form a part of the sacs. The walls were represented by mucous membrane and muscularis mucosae.

The muscularis of the intestinal tract near the diverticula and at some distance from them was thin and showed considerable fatty degeneration. Fat was deposited in fine droplets throughout many muscle fibers. Sections were treated with potassium bichromate after the method of Bell,¹² to fix the fat, and paraffine sections were stained with Sharlach R.

The small diverticula of the transverse colon were likewise protrusions of the mucous membrane through the muscularis. No special relationship of blood vessels to these diverticula was established.

It is of interest to note the presence of marked chronic interstitial pancreatitis and slight periportal cirrhosis of the liver in

¹² Bell, E. T., *Jour. Path. and Bacteriology*, vol. 19, 1914, p. 105.

association with the diverticulum at the major papilla. Distention of this diverticulum would have exerted pressure on the common bile duct and pancreatic duct. Much significance, however, cannot be attached to this relation in view of the associated generalized sclerosis.

The two upper diverticula of the last case were obscured by folds of mucous membrane and were found only after special search was made. It seems probable that closer observation would show that diverticula of the duodenum occur more frequently than former observations have suggested.

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Resumen por G. Carl Huber, por el autor Tanzo Yoshinaga.

Contribución al estudio del desarrollo temprano del corazón de los mamíferos, con especial mención del del conejillo de Indias.

Las observaciones publicadas en el presente trabajo se basan en el estudio de una serie de estados muy próximos en el desarrollo de los embriones del conejillo de Indias, cortados en serie de 5 a 10 μ de espesor, tanto en el plano sagital como en el transverso. Los estados estudiados comprenden el periodo de desarrollo desde el duodécimo al décimo-quinto día después de la inseminación, o sea desde el momento en que aparecen por primera vez los angioblastos en la región más tarde ocupada por el corazón hasta el estado de corazón en forma de S. Los estados muy próximos han sido reconstruidos en placas de cera mediante el método de Born, y el estado más joven presenta el comienzo de la formación del espacio pericárdico.

Las pruebas obtenidas mediante este estudio son interpretadas por el autor como una demostración de que los angioblastos que han de formar el futuro corazón tubular endotelial derivan independientemente del mesodermo de la esplanenopleura. El esbozo de los pliegues miocárdicos y su desarrollo progresivo son objeto de una discusión por parte del autor, quien presenta su desarrollo en una serie de figuras basadas en reconstrucciones.

Translation by José F. Nonidez
Cornell Medical College, New York

A CONTRIBUTION TO THE EARLY DEVELOPMENT OF THE HEART IN MAMMALIA, WITH SPECIAL REFERENCE TO THE GUINEA PIG

TANZO YOSHINAGA

Department of Anatomy, University of Michigan

TWENTY-THREE FIGURES

INTRODUCTION

Since the fundamental investigations on the development of the heart in mammals by His, Born, and others were published, many prominent investigators have contributed to our knowledge concerning the earlier stages of development of the heart and of the pericardial cavity in representatives of almost all classes of vertebrates. The earlier workers began their investigations after the developmental stage in which the embryonal heart tube had already assumed the complete S form. From the results of their work, however, only very general conclusions can be drawn. In many important details the literature shows contradictions, while each theory advanced has been supported by investigators of recognized ability.

The following work was undertaken at the suggestion of Professor Huber. I take this opportunity to express my hearty thanks to him for the use of his collection, which he placed at my disposal, and for his invaluable help. This work concerns itself chiefly with the origin of the endothelial tubes, the early development of the pericardial cavity, and the mode of confluence of the bilateral myocardial tubes, written with the hope of contributing something of interest to the early development of the heart.

The material for this study was obtained from many uninterrupted series of embryos of the guinea pig, from the embryological

collection in the Department of Anatomy of the University of Michigan, prepared by Professor Huber. The great majority of the embryos were fixed in Carnoy's fluid and all were cut into either cross or sagittal series at either 5μ or 7μ thickness, also a few at 10μ thickness.

In a study of the development of the endothelial and the myocardial tubes and of the pericardial cavity, to show their successive developmental changes and their relative topographical relations, a number of models were prepared according to Born's method of wax-plate reconstruction. These were made at a magnification of 300 diameters, with the aid of the projection apparatus, by superimposing the drawings of every section of the cranial part of the embryo. The thickness of the wax plates was changed in proportion to that of the sections from 1.5 to 2.1 and 3 mm., respectively. All the models described here are deposited in the Department of Anatomy at the University of Michigan.

All the figures of the sections presented here were drawn at relatively high magnifications with the aid of the camera lucida and subsequently reduced to the proper size in reproduction. The figures of the models were prepared according to the method described by Doctor Atwell. I am grateful to Doctor Guild and to Mr. Smith for help in preparing the wax plates.

LITERATURE REVIEW

Before considering the material studied in this investigation, I wish to refer to some of the theories held by earlier writers. I do not wish to give a complete résumé of the historical developments, but rather briefly to indicate the representative opinions directly concerning the subject.

In the embryonic shield of mammals at first there are present two pairs of longitudinal vessels, one of them situated near the middle line on the entoderm on each side representing the supra-intestinal longitudinal blood vessels, and the others are found primarily on the entoderm approaching the lateral margins of the embryonic shield and these representing the subintestinal longi-

tudinal blood vessels. In the cephalic portion of these latter the heart is formed, the walls of these vascular Anlagen being peculiarly dilated and thickened. From this ontogenetical standpoint, the early development of the heart is merely a part of the development of the intra-embryonic vascular system.

In the survey of the literature on the subject of the origin of the intra-embryonic blood vessels in mammals, the theories held by the earlier investigators, and still maintained, can be divided into three categories.

The first theory was advanced by His, who made his observations of the flat embryonic shield of the chick. Hertwig, Kölliker, and others supported this theory. According to the *Einwachsungslehre* of His, with which the theory of the parablast was first connected, the early blood vessels of the embryonic shield are formed by a sprouting or ingrowth of the preexisting endothelial lining of the blood vessels, which had previously developed in the extra-embryonic vascular area. The ingrowth of the blood-vessel Anlagen into the embryonic area takes place as solid, tenuous sprouts of the cells, which are primarily found to be anastomosed with each other, to form a close network in the area pellucida.

These sprouts of cells enter into the embryonic area through the space between the splanchnopleure and entoderm, until they reach the somitic region, where they anastomose and become canalized to form hollow vessels. Ultimately, this network of the endothelial lining forms the dorsal aortae, uniting in a longitudinal direction. The ingrowth of the endothelial sprouts is not limited to the somitic portion, but takes place also in the cranial part, entering from the lateral margin of the embryonic shield, through the space between the splanchnopleure and the entoderm, until they reach the heart Anlage, where they develop the endocardium. The endothelial sprouts spread out, forming the ventral aortae and the blood vessels of the cranial region. The former blood vessels will be connected with the dorsal aortae which are prolonged cranialward by the sprouting and in the same way by turning over of the blind end of the pharynx ventralward.

Türsting studied the development of the aortae in the rabbit and confirmed the conclusion of His, namely, that these vessels are

formed by a longitudinal anastomosis of the ingrown endothelial sprouts, derived from the extra embryonic area, through the space between the splanchnopleure and the entoderm. He noticed the early connection of the dorsal aortae with the vitelline plexus.

Vialleton and Evans studied the development of the intraembryonic blood vessels in birds and came to the conclusion that in birds the greater part of the descending aortae is developed by a conversion into a continuation of the aortae of the innermost strand of the capillary plexus, extended into the embryonic shield from the neighboring yolk sac.

Lewis investigated the intraembryonic blood vessels in rabbits from eight and one-half to thirteen days after insemination, and claims that from the network of vessels in the splanchnopleure of the yolk sac all intraembryonic vessels are apparently derived as offshoots. The network ends mesially in two aortae. With the formation of the pharynx, this net is so folded as to produce dorsal and ventral aortae with the connecting first aortic arch.

Bremer recently repeated the investigation of the same material and came to practically the same results. His summary is given as follows: "In the rabbit, the dorsal aorta, the first aortic arch, the conus arteriosus, and the lateral heart are all parts of an original network of angioblast cords, derived from the extraembryonic plexus of blood vessels. Those portions of the network which are mechanically favored in their position persist, the other portions disappear. Although dealing in this paper with the development as found in rabbit embryos, I have examined various other species, as the chick, pig, sheep, etc., and feel satisfied that in all essential points the story of the development of these primary vessels in other vertebrates will be found similar to that here described."

Many other investigators support this theory, while still others do not accept it. Ranvier seems to think that the *Einwachsungslehre* of His must be regarded as a simple hypothesis, and states: "Mais il est clair qu'aucun embryologiste n'a pu suivre ce développement continu par bourgeonnement dans le corps même de l'embryon; c'est là une simple hypothèse."

The second theory of embryonic vasculogenesis was first advanced by Rabl, who asserts that the first aortic arch in amphibia

is formed by the accretion and extension of the endothelial cells proliferated in the paired heart rudiments, when these endothelial tubes were developed. Moreover, he applies this possibility to other blood vessels of the embryo, in which they are formed by the extension of the preexisting endothelial cells, as, for instance, can be seen in the regeneration of the capillaries. His statement reads as follows: "Die Beobachtung, dass bei den Amphibien die ersten Aorten Bogen durch Auswachsen des Endothelsäckchen entstehen, legt uns aber noch die Frage nach, ob es vielleicht auch das Endothel aller anderen Gefässe in letzter Instanz auf die Zellen des Endothel-säckchens zuruckzuführen sei, mit anderen Worten ob nicht vielleicht all Gefässe in derselben oder in ähnlicher Weisse entstehen, wie die Capillaren."

Furthermore, in his later work, "The Theory of the Mesoderm," he repeated his assertion: "Ich habe die erste Entwicklung der Gefässe namentlich an den Aorten wiederholt genau verfolgt, und ist mir kein Fall erinnerlich, der mich an der Ueberzeugung irre gemacht hätte, dass neue Endothelen immer nur aus bereits bestehenden ihren Ursprung nehmen."

Rückert investigated the early development in the eggs of selachians, in which at first the subintestinal veins can be seen in the anterior embryonic shield. Here the endothelial cells are produced from the ventral angles of the lateral plates, detaching as free angioblasts, which subsequently assume forms of the cellular groups or chains between the splanchnopleure and entoderm. With regard to the anlage of the aortae in the anterior part of the embryonic shield, Rückert assumes still further that their origin is in loco and that here are anticipated the adjacent mesodermal somites, subordinately the dorsal wall of the gut comes under consideration.

P. Mayer discovered the transverse blood vessels in the eggs of the torpedo, connecting the subintestinal veins with the aortae, and he attributed their origin to the emigrated cells from the ventral parts of the mesoderm, wandering along the gut wall dorsalward. Rückert agreed with the opinion of Mayer. In the vascular development of the embryo he claimed that the angioblasts still arise in loco, for example, the blood vessels of the pronephros

from the visceral walls of the somite. The angioblasts of the mandibular blood vessels from the visceral plate of the second cranial somite as well as from the wall of the foregut.

Raffaele, Emmert, and others have accepted the local formation of the embryonic blood vessels and are in agreement with the idea of Rückert. After the first publication of his work in 1888, Rückert investigated all classes of vertebrates and confirms his claims to the local formation of the embryonic blood vessels. Concerning the development of the chick, he expressed himself as follows: "So findet also beim Huhn statt des Einwachsens der Gefässanlagen eine von der Peripherie des Keimwalles gegen den Embryo zu fortschreitende Differenzierung derselben aus dem Mesoderm statt, was mit den Beobachtungen an Selachieren übereinstimmt. Auch innerhalb des Embryo entstehen beim Huhn die Gefässanlagen durch locale Ausschaltung des Zellen Materials aus dem Mesoblast, wovon ich mich bei der Herz so wie der Aortenbildung überzeugt habe."

Mollier reached the same conclusion, confirming Rückert. After thoroughly studying the material of a wide scope, he concludes as follows: "Es lässt sich also für die Genese der embryonalen Gefässe der Amnioten zur Zeit ein Urteil dahin fassen, dass die Lehre von der localen Entstehung der Gefässzellen auch hier Geltung besitzt und dass die von His und Vailleton gegebenen Flächenbilder, ferner die Rekonstruktionsbilder von Türstig in dem Sinne zu deuten sind, dass die im Embryo sichtbaren ersten Gefässzellenstränge nicht als Sprossen ausserembryonaler Gefässanlagen entstanden sind, sondern vielmehr ihre Entstehung aus einzelnen, in loco entstandenen und netzförmig vereinigten Gefässzellen nehmen."

Sobatta not only supported the theory of Mayer and Rückert, but he also emphasized that those blood vessels found on the walls of the yolk sac were derived from the intraembryonic blood vessels as a result of their continuous outgrowth.

New light was shed on this contradictory evidence by a number of investigators who employed the methods of experimental embryology and were able to show that the yolk-sac angioblasts may be kept out of communication with the intraembryonic blood

vessels, thus lending evidence in favor of the local formation of the embryonal blood vessels.

The mechanical separation of the vessels of these two portions was employed by Gräper, Hahn, Miller and McWhorter. These workers have obtained endothelium on both sides of the chick embryo, in which one side was severed from the extraembryonic blastoderm. A further strengthening of the theory of the local formation is found in the recent experimental work by Reagan, who writes as follows: "In conclusion it is well to consider the following recently established facts which should share in defining our morphological interpretation. The yolk sac is not necessarily the site of formation of the earliest blood vessels. Intraembryonic blood vessels develop *in situ* when communication of the extraembryonic vessels with intraembryonic tissues is prevented by chemical or mechanical means."

The method of exposing the developing embryo to diluted anesthetics was employed by Stockard, who claimed as a result of his work that in *Fundulus* embryos the heart endothelium and the aorta arise *in loco* within the embryo, as the blood vessels, even the mesoderm are absent in the yolk sac in the cranial portion.

Among the investigators who have accepted the local formation of the intraembryonic blood vessels, opinions are still quite divergent at present as to from what part of the embryonal blastoderm the angioblasts are differentiated. I shall not again thoroughly discuss this point, as I have already done so in my previous paper on this subject. But I shall add that there are many authors who consider the origin of the angioblasts in mammals as derived from the entoderm. Martin figures a cross-section of a cat embryo 2 mm. long and in his prominent "Lehrbuch der Anatomie der Haustiere" states: Während man über die erste Entwicklung der Blutgefäße im Embryonalleib noch nicht im Klaren ist, kennt man die Bildung der peripheren Gefäße genau. Ihrer Abstammung nach sind die Innenwand (endothelium) auch der embryonalen Gefäße wie die des Herzens entodermaler Natur, während die übrige Wand vom Mesoderm geliefert wird. Ich finde bei Katze die erste gefäßbildenden Zellen und Zellgruppen eininnigsten Zusammenhand mit dem Entoderm des Darmes, ja so

gar schon rundliche Spaltbildungen in Entoderm selbst. Auch die ausserembryonalen Blutgefäße sind nach Rabl entodermalen Ursprungs." In the figure depicted by Martin, angioblasts can be seen, connected with both the mesoderm and the entoderm.

In the study of a perameles embryo of 6.08 mm. in length, Miss Parker says with regard to the origin of the endothelial cells: "The evidence of this stage does not justify any definite statement with regard to the origin of the endothelium of the heart." And, moreover, she adds, that in an earlier stage the appearance by no means excludes the possibility of the entodermal origin of the endothelium.

After investigating the origin of the endothelial and blood cells in the embryo of the ferret, Wang comes to the following conclusion: "The facts revealed by the study of the early stages in the development of the ferret point to the conclusion that, whilst blood cells and vascular endothelium are closely related to each other and are formed invariably between the mesoderm and entoderm, there is evidence to show that, in the ferret, the origins of these two vascular elements are separate and distinct—the blood cells arising from the entoderm and the vascular endothelium from the mesoderm."

"If the biphyletic origin of the blood cells and vascular endothelium is to be accepted, two more points still remain to be solved, namely, how, when, and where the first blood cells enter the circulation. Unfortunately, the ferret embryos, at present worked upon, provide no definite evidence on this point, but it is quite clear that angioblast cells are formed outside the embryonic area, and that blood vessels are formed inside the embryonic area, and are at first devoid of blood corpuscles."

We shall now briefly review the data concerning the mode of the fusion of the bilateral heart anlagen; there are many divergent opinions.

Since Hensen first declared that in mammals the heart anlage is bilateral, one on each side of the embryo not far from the mid-sagittal plane and on the ventral aspect of the pericardial cavity, it was long believed that these two lateral heart anlagen ultimately came in contact and fused together in the middle ventral

surface of the embryonic shield, forming a single secondary heart tube. Even though the secondary fusion of the bilateral endothelial tubes is universally acknowledged, there are many contradictory theories concerning the formation of the single myocardial heart anlage together with the mode of fusion of the bilateral primitive pericardial cavity. In his work on comparative embryology, Balfour thus speaks: "In mammals the two tubes out of which the heart is formed, appear at the sides of the cephalic plates, opposite the region of the mid and hind brain. They arise at a time when the lateral folds which form the ventral walls of the heart, are only just becoming visible. On the formation of the lateral folds of the splanchnic walls, the two halves of the heart become carried inwards and downwards, and eventually meet on the ventral side of the throat. For a short time they here remain distinct, but soon coalesce into a single tube."

Minot writes: "In mammals by the bending down of the layers and the expansion of the coelom the vorderdarm is shut off and the lateral heartanlagen are brought together in the median line below the vorderdarm, and there fuse into a single thick tubular wall around the double endothelial heart; it is not long, however, before the endothelial tubes also fuse into one. As in the chick the two mesothelia, when the median heart arises, form a membrane (mesocardium) by which the heart is attached to the tissue above and below; both mesocardial membranes break through, putting the two coelematic cavities into communication and leaving the tubular heart suspended by its ends."

The theory of the fusion of the lateral folds of the splanchnic walls, enclosed within the laterally placed pericardial cavities at the ventral side of the foregut in mammals, as in amphibia or birds, as many investigators have asserted and now believe, is supported by the following authorities: Balfour ('81), Strahl and Carius ('89), Tandler ('12), Wilson ('14), Bryce ('08), Minot ('92), Bailey ('12), Schultze ('15), Dandy ('10), Martin ('02), and Arey ('17). From this it would seem that, as many of the above named authors claim, the heart must be provided, at least temporarily, with a ventral and a dorsal mesocardium.

In a similar way in his valuable work on the first heart anlage, Mollier says: "Das Mesocard, ventral oder medial gelegen, ist dem dorsalen der Anamnier zu vergleichen. Ein dem ventralen entsprechendes kann erst nach dem Zusammenstossen beider Pleuro-pericardialhöhlenwände gebildet werden. Beim Meer-schweinchen hingegen liegen die ersten Herzzellenstränge lateral von den Firsten der Darmfalten, und sie werden durch den Darm-schluss gar nicht unter die ventrale Darmwand verlagert, wie beim Kaninchen, sondern rücken, zwischen dorsaler und ventraler Mesocardfalte gelegen, einander näher, bis zur Berührung und endlich Verschmelzung. Doch erfolgt auch hier, wie aus der Figur ersichtlich, der Durchbruch des ventralem Herzgekröses zuerst."

Robinson has pointed out that the formation of the foregut is mainly attributed to the unproportionately rapid development of the embryo over the relatively stationary line between the embryonal and extraembryonal areas. If the idea were true, that lateral folds of the mesoderm converge ventrally until their entodermal covering has met together in the ventral middle line and both lateral pericardial cavities have fused together beneath the ventral walls of the foregut, then the heart is not only attached by the dorsal mesocardium to the foregut, but also by the ventral mesocardium to the ventral wall of the body. However, Robinson denies this generally accepted idea and the existence of the ventral mesocardium absolutely, at any time in the development of mammals, and he applies this fact to support his theory, that the separation of the foregut from the yolk sac is not due to the tenaciously held process of the tucking in of the margins of the embryonic area, but to the fact that the relatively slow-growing margin is demarcated between the embryonic and extraembryonic portions of the wall of the ovum, which rapidly increase their extent, expanding over the boundary margin. According to him, in mammals, the pericardial mesoderm is present in the pericardial portion of the embryonic area, and it is completely separated into somatic and splanchnic layers before the head fold appears. There is therefore a single pericardial cavity which extends from side to side along the anterior boundary of the embryonic shield. As the head fold is formed, the pericardial region

is reversed and it is carried into the ventral wall of the foregut, where it is present as a U-shaped tube, which communicates with the general coelom at each end. The rudiments of the heart are formed in the splanchnic layer of the pericardial mesoderm. Therefore, after the reversal of the pericardial area, they lie on the dorsal wall of the pericardial cavity, attached to the ventral wall of the foregut by the dorsal mesocardium.

Prior to Robinson, Ravn pointed out the correlation between the formation of the foregut and the forward growth of the embryonic shield. He also thoroughly described the mode of reversal in the primitive pericardial cavity.

On the other hand, many investigators believe that an active backward progression of the foregut opening occurs, in addition to the forward growth of the head fold. They deny the actual fusion of the lateral mesodermal folds in the ventral middle line. Rouviere agrees with Robinson as to the absence of the ventral mesocardium in mammals, while he does not dismiss the meaning of the forward growth of the head fold on the formation of the foregut. According to his account, in a rabbit embryo of 201 hours, both the pericardial cavities (*les deux cavités pariétales*) show the separated bilateral canal on each side, which has grown forward around the anterior end of the head fold and become fused together from a single continuous cavity in the embryo of 207 hours. The splanchnopleure forming the caudal wall of the pericardial cavity assumes a continuous fold, which Tourneux designated as the *cardiac fold* (*repli cardiaque*) and which he considered as growing backward automatically as a whole. As the free edge of the splanchnopleural fold has progressed always in advance of the primordial heart, no fusion of the splanchnopleure is involved and also no ventral mesocardium is formed.

In a description of the growing processes in the developing chick embryo, having kept them under direct observation while still alive, Gräper asserts that there is considerable evidence in support of the view that the margin of the foregut (*Darmpforte*) moves caudally, concurrently, with the forward growth of the head fold. Moreover, he marked out diagrammatically the mode of the backward progress of the foregut opening and a quite different manner of the closure of the foregut than that of the medullary canal.

Uskow also claims the automatic backward progress of the foregut opening, according to the increase of the pericardial cavity.

In her study of the early stages in the development of marsupials, Miss Parker declares that the forward growth of the head fold doubtless plays an important part in the initiation of the formation of the foregut and that the actual backward growth of the foregut opening, but not the fusion of the lateral folds, brings about the lengthening of the foregut.

In the study of the early development of the heart and cranial blood vessels in ferret embryos, Wang agrees with Miss Parker in the absence of the ventral mesocardium, there being no fusion of this part of the pleuropericardial wall, nor that any part of the gut closure is effected by the fusion of the lateral folds.

OBSERVATIONS

Stage I

The material for this stage consists of many specimens removed from the uterus of the guinea pig thirteen days and twelve hours or fourteen days and eleven hours, respectively, after insemination. Some of them were cut longitudinally and the others transversely.

A. The first embryonic shield which came under consideration, was removed from the uterus thirteen days and twelve hours after insemination and was in a cross-section having a 7μ thickness; 233 sections fell to the embryonic shield. The head fold had not begun to develop. The shallow neural groove was present on the surface of the embryo. The primitive streak was well developed and terminated caudally in a shallow notched groove. The mesoderm was thickened in the caudal part of the embryonic area and indicated the allantoic mesoderm. In the notochord there was present the chordal canal at its caudal end, but in other parts it was spread out to form a chordal plate through dehiscence of the ventral wall. The caudal end of the notochord was fused with an area of ectodermal proliferation at the cranial end of the primitive streak.

In the mesoderm there was observed no evidence of mesodermic somites nor could the coelomic cavity be detected. The mesoderm consisted of two lateral wings on each side in the cross-section, separated completely in the middle line by the notochord, except in the cephalad end of the embryonic shield and in the region of the primitive streak. In the cranial end of the embryonic shield, that is, the part distal to the future pharyngeal membrane, in which the ectoderm and entoderm were coalesced, the lateral wings of the mesoderm were fused, continuing caudally into the lateral wings, but sharply terminated against the extraembryonic area cranially and laterally. In this portion of the mesoderm, namely, in the pericephalic mesoderm, it formed a thinner layer than anywhere else. In the primitive streak a large mass of undifferentiated mesodermal cells was fused with both lateral wings, obliterating the demarcation between the mesoderm and ectoderm. In the mesoderm two layers of the cell band could not be distinguished; the dorsal surface of the mesoderm was, in general, compact, its outline was clear-cut; here the spindle-shaped nuclei had a relatively regular arrangement.

Between the dorsal surface of the mesoderm and ectoderm, as well as between the ventral surface of the mesoderm and entoderm, there could be distinguished clear intervals, which could be attributed in large measure to shrinkage. The mesodermal cells were spread out in two or three layers and were spindle-shaped and connected with each other by short protoplasmic processes. In the ventral surface of the mesoderm a loosening of the cell band could be seen, characterized by an increased distance between the respective nuclei. Inter-cellular spaces became more distinct and wider, the spindle-shaped nuclei had no definite arrangement. In nearly all sections there could be demonstrated some free, isolated cells, detached from the cell band of the mesoderm, lying between the ventral surface of the mesoderm and entoderm, as shown in figure 1. According to His, these cells are identical with the angioblasts. In some sections the angioblasts are connected with the indented ventral margin of the mesoderm by broad protoplasmic bridges;

in some other sections the protoplasmic bridges are narrow, the cells are distinctly pedunculated; in still other sections they are joined to the mesoderm by faint fibrils. Frequently a mitotic figure can be recognized in the mesodermal cells, adjacent to the angioblasts. These findings show that there can be no doubt of a distinct proliferative activity of the mesodermal cells; furthermore, every transitional feature of the migration or the detachment of the mesodermal cells, which apparently are destined to become angioblasts, point out the fact that these angioblasts have originated in the ventral surface of the mesoderm.

Figure 1 was reproduced from the forty-ninth section, counting from the cephalic border of the embryonic shield, and

LEGEND LETTERS FOR ALL THE FIGURES

<i>A.</i> , atrium	<i>F.G.O.</i> , foregut opening
<i>Am.</i> , amnion	<i>I.M.S.</i> , intermesocardial space
<i>Ang.</i> , angioblast	<i>L.P.C.</i> , lateral pericardial cavity
<i>Ao.</i> , aorta	<i>M.C.</i> , myocardial cavity
<i>A.V.C.</i> , atrioventricular constriction	<i>Mes.</i> , mesoderm
<i>A.V.Ca.</i> , atrioventricular canal	<i>M.G.</i> , medullary groove
<i>B.</i> , bulbus cordis	<i>M.T.</i> , myocardial tube
<i>B.V.C.</i> , bulboventricular constriction	<i>N.</i> , notochord
<i>C.M.L.</i> , craniomedian limb of the pericardial cavity	<i>P.</i> , pericardium
<i>Co.</i> , coelom	<i>P.C.</i> , pericardial cavity
<i>D.M.</i> , dorsal mesocardium	<i>P.M.</i> , pharyngeal membrane
<i>D.W.P.</i> , dorsal wall of pericardium	<i>P.P.P.</i> , pleuropericardial passage
<i>Ect.</i> , ectoderm	<i>S.A.C.</i> , sino-atrial constriction
<i>End.</i> , endothelium	<i>Som.</i> , somatopleure
<i>Ent.</i> , entoderm	<i>Spl.</i> , splanchnopleure
<i>E.O.</i> , endothelial offshoot	<i>S.R.</i> , septum ridge
<i>E.T.</i> , endothelial tube	<i>S.V.</i> , sinus venosus
<i>F.G.</i> , foregut	<i>T.A.</i> , truncus arteriosus
	<i>V.W.F.</i> , ventral wall of the foregut

Fig. 1 The 49th section of a series of 233 cross-sections of $7\ \mu$ thickness of an embryonic shield of the guinea pig, removed 13 days 12 hours after insemination. The early stage of the formation of the angioblasts from the ventral surface of the mesoderm. $\times 150$.

Fig. 2 The 41st section of a series of 237 cross-sections having a $7\ \mu$ thickness, removed from the uterus of a guinea pig 14 days 11 hours after insemination. The ventral surface of the mesoderm becomes loosened and angioblasts are separated. $\times 150$.

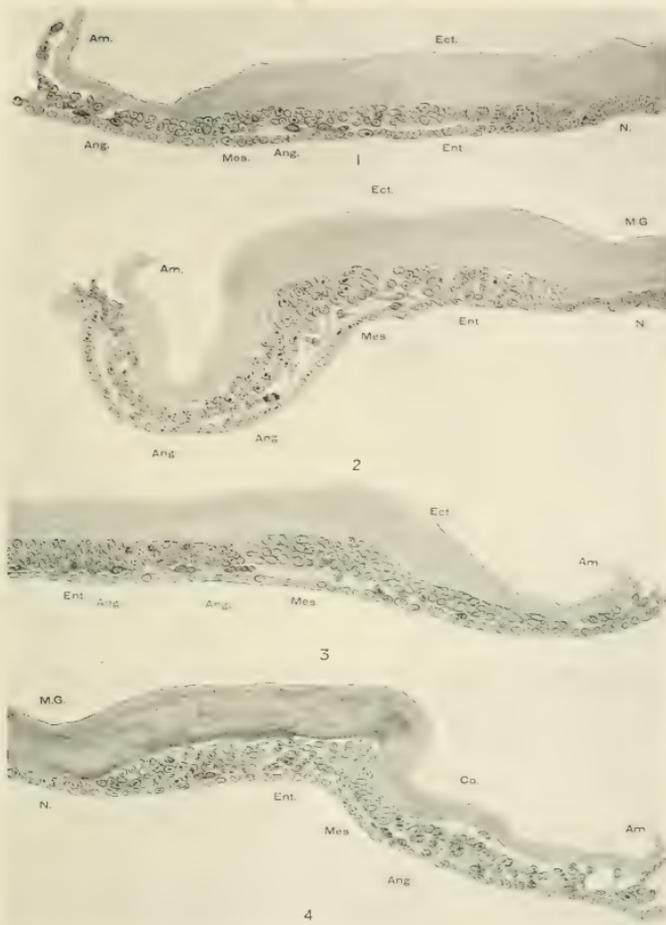


Fig. 3 The 40th section of a series of 162 sagittal sections having a $7\ \mu$ thickness. This embryonic shield was removed from the uterus of a guinea pig 13 days 12 hours after insemination. The angioblasts form the cell strands between the splanchnopleura and endoderm. $\times 150$.

Fig. 4 The 63rd section of a series of 300 cross-sections of $7\ \mu$ thickness of an embryonic shield of a guinea pig, removed 13 days 11 hours after insemination. In the lateral plate of mesoderm the discontinuous coelom is present. $\times 150$.

corresponds approximately to the future hindbrain region, in which region endothelial tubes had been differentiated from the angioblasts in the next stage. As the contour of the entoderm is distinctly demarcated from these cells in our specimens, as the figure shows, it may be excluded from direct participation in the formation of these cells.

B. The next embryonic shield selected for discussion was removed from the uterus of a guinea-pig fourteen days and eleven hours after insemination. It is cut in cross-sections, having a 7μ thickness, and 237 sections fall to the embryonic area. The flat incipient head fold has begun to develop, marking definitely the cranial margin of the neural plate. In this portion the ectoderm has been elevated above the surrounding embryonic shield. A broad and shallow neural groove, which gradually narrows cephalad, is present on the surface of the embryo. The primitive streak is well distinguished on the embryonic surface as of a transitional portion from the cranial neural groove into the caudal primitive groove. The notochord has acquired a tubular form at its caudal end in some more anteriorly placed portions, but in the majority of the sections its ventral wall is opened into the yolk sac, to form the chordal plate. The caudal end of the notochord is fused to the cranial part of the primitive streak. No mesodermal somites are recognized nor can any indication of the coelomic cavity be detected.

Figure 2 was reproduced from the forty-first section, counting from the cephalic amnion attachment. The mesodermal layer is thicker than that found in the preceding specimen; this is due partly to the numerical increase of its component cells and partly due to a loosening of the arrangement of the cells. In the mesoderm no layers can be distinguished, the spindle-shaped cells having no definite arrangement. The loosening of the cell is more readily demonstrable on the ventral surface of the mesoderm; its ventral outline forms a zigzag contour, due to the shorter or longer protoplasmic processes which are seen extended from the ventral row of the mesodermal cells toward the underlying space. Moreover, as can be pointed out in the figure, some cells are projected into the underlying space beyond

their surrounding group of cells, while others present a mitotic figure and have their axis directed to the space under the mesoderm and are pedunculated into the underlying space, but remain connected with the mesodermal layer by means of their narrow protoplasmic bridge. There can be seen a few spindle-shaped cells, which appear completely detached from the mesoderm and lie scattered between the mesoderm and entoderm. The area of the distribution of these cells, which we regard as angioblasts, more numerous in this embryo, extends over a wider range than is observed in the previous embryo. A glance at the figure will prove that the origin of these cells is derived from the mesoderm of the splanchnopleure.

In figure 3 there is presented a drawing of a sagittal section of an embryonic shield of approximately the same stage of development as that described under figure 2.

This series belongs to an embryonic shield of a guinea pig, removed from the uterus thirteen days and twelve hours after insemination. It includes 162 sections, having a 7μ thickness.

The figure was reproduced from a drawing of the fortieth section, counting from the lateral amnion attachment. This section passed through the flat head fold near its lateral margin. The line of sectioning in this series was almost parallel to the mid axis of the embryonic shield. A study of the series shows that the developmental stage is just prior to the formation of the first mesodermal somite, which is indicated but not completely formed. The primitive streak extends approximately a third of the length of the embryonic shield. The general finding of the mesoderm is similar to that of the foregoing specimen. In the midsagittal plane the cranial end of the chordal plate terminates insensibly in the entoderm, where the pharyngeal membrane will be recognized. Cephalad to this membrane the pericephalic mesoderm is observed; its cranial limit terminates freely at the cranial amnion attachment. This pericephalic mesoderm is continued into both mesodermal wings caudolaterally. The ventral surface of the mesoderm is loosened and presents a coarse appearance. Between the ventral surface of the mesoderm and the continuous layer of ectoderm angio-

blasts can be seen forming cell strands, ranging one after another, approximately parallel to the long axis. In the cross-section these cell strands may be shown as single cut cells. The faintly stained protoplasmic processes or slightly rotated, tenuous protoplasmic fibrils are given off from the surface of the cell strands. Some of these anastomose with each other and others are connected with the cells of the adjacent mesoderm. In this fashion their protoplasmic fibrils form a kind of feltwork between the mesoderm and entoderm. In brief, it is only a repetition of the processes which produce the angioblasts from the mesoderm of the splanchnopleure, as observed in the preceding embryo, but the angioblasts are a step further differentiated.

C. In figure 4 there is presented a cross-section drawing of an embryonic shield of a guinea pig, removed from the uterus thirteen days and eleven hours after insemination. This series includes 300 sections, having a 7μ thickness. In actuality this embryo presents only a slight advance in development over that discussed under figures 2 and 3.

The first mesodermic somite is indicated, but not completely formed. The intraembryonic coelomic space, which may be regarded as the future primitive pericardial cavity, considering its topographic position, shows simply a beginning of a very narrow cleavage in the lateral mesoderm of the cranial portion of the embryo. In some sections the two layers of the lateral plate of the mesoderm are separated from each other, and there can be seen narrow, discontinuous clefts between the mesodermic layers, while in other sections the whole mesoderm remains apparently solid. As a transition of these two extremes, in still other sections the coelomic space is shown as little more than a lineal cleavage. In brief, the coelomic cavity is forming from multiple foci and is not connected with the extraembryonic coelom. In the pericephalic mesoderm no coelomic space can be seen.

The figure reproduces a drawing from the sixty-third section, in which there can be seen an irregularly outlined splanchnopleure, from a relatively clear-cut contour of the somatopleure, separated by an incipient slip of the coelomic space. The

splanchnopleure shows a slightly thicker layer of spindle-shaped cells: in some of them mitotic figures are present, indicating a proliferative activity. A number of angioblasts are scattered singly, while some others are grouped in a flat strand between the ventral surface of the mesoderm and entoderm.

Practically the same stage of development as that described in figure 4 can be seen in an embryonic shield, removed from the uterus fourteen days and four hours after insemination.

In figure five we see a drawing of the thirty-second section, counting from the lateral amnion attachment. This series, cut in the sagittal plane, includes 156 sections, having a 7μ thickness. This section passes through the well upwardly projected head fold near its uplifted lateral margin. Under this head fold there can be recognized five discontinuous coelom spaces in the lateral plate of the mesoderm, each of which is interrupted by a substantial bridge. The mesodermal cell layer of the splanchnopleure is distinctly thickened and loosened. The spindle-shaped mesodermic cells assume a somewhat irregular arrangement. A number of them have disposed themselves in such a direction that their long axis is vertical to the ventral surface. A number of angioblasts are scattered under the mesoderm of the splanchnopleure and some of them are connected with this layer by their protoplasmic processes. Mitotic figures, seen in some of the mesodermal cells, show their proliferative activity. In some of the sections the discontinuous coelomic space can be seen in the pericephalic mesoderm, but it entirely disappears as it approaches the midsagittal plane, where the mesodermic layer has remained still in a solid condition, as can be found in figure 5B. In this respect this embryonic shield differs from that shown by Robinson and of several other workers. Robinson says that in mammals the mesodermic layer extends through the pericardial portion and is cleft into somatic and splanchnic layers before the head fold is formed. In our specimens the intraembryonic coelomic space is present discontinuously in the cranial region of the embryonic shield and totally absent as it approaches the middle plane of the pericephalic mesoderm, even though the head fold is already formed.

Stage II

The material on which the following description of stage II is based consists of several embryos, certain of which are cut transversely and others longitudinally.

A. This specimen was removed from the uterus of a guinea pig fourteen days and eleven hours after insemination. The series includes 307 cross-sections, having a $7\ \mu$ thickness. A plastic reconstruction of the cranial portion was made with the Born method, and the whole shield, reconstructed for another purpose, was used for this study. This embryonic shield, as the model shows, presents a flat head fold, which is slightly more elevated than in the previous stage. The head fold can be divided into two primary parts. The cranial part is long and wide and projects over the cranial and lateral walls of the cranial body elevation. The caudal part is small and passes insensibly into the spinal portion. There is present a well-developed medullary groove in the cranial portion of the embryonic shield and its caudal end becomes gradually shallower until it disappears at the primitive streak in the caudal fourth of the embryonic shield. Its cranial end is terminated near the cranial extremity of the head fold. The deepest portion of the medullary groove corresponds to the region of the hindbrain plate. A well-marked anlage of the trigeminus is present as a thickening of the ectoderm. There are present three pairs

Fig. 5A The 32nd section of a series of 156 sagittal sections of $7\ \mu$ thickness of an embryonic shield of a guinea pig, removed 14 days 4 hours after insemination. The mesoderm is thickened and loosened, a number of discontinuous coelomic spaces is present, angioblasts are being produced from the splanchnopleura. $\times 150$.

Fig. 5B The 77th section of the same series from which figure 5 was drawn, passing through practically parallel to the midsagittal line. The pericephalic mesoderm shows a relatively thinner layer than elsewhere and lies anterior to the primitive pharyngeal membrane, as the foregut has not yet developed. In accordance therewith, the reversal of the preumbilical region of the embryonic body does not occur. $\times 150$.

Fig. 6 The 87th section of a series of 307 cross-sections of $7\ \mu$ thickness of an embryonic shield of a guinea pig, removed 14 days 11 hours after insemination. The pericardial cavity opens widely, under the thickened splanchnopleura the endothelial tube is first formed in this embryo. $\times 150$.



Fig. 7 The 27th section of the same series from which figure 6 was drawn, embracing the nearly anterior margin of the head fold. The pericephalic mesoderm separates into two layers, by the lineal coelem space, which continues into the lateral pericardial cavity. $\times 150$.

of mesodermic somites with a fourth pair forming. The foregut has begun to develop in the embryo; for the length of five sections its lumen is invaginated upward into the head fold.

Both the lateral primitive pericardial cavities are presented in the lateral plate of the mesoderm (fig. 6), which is completely separated into two lateral wings by the notochord, except in the cranial end of the embryonic shield, namely, beneath the cranial extremity of the head fold and in the region of the primitive streak. In these places two lateral wings come to fusion. In the mesoderm, which is produced by the fusion of both the lateral mesodermic wings in the middle line, beneath the cranial extremity of the head fold, in front of the pharyngeal membrane, a lineal cleavage can be recognized (fig. 7) by which the mesoderm is separated into two distinct layers. This coelomic space in the pericephalic mesoderm is formed by a forward extension of both the lateral primitive pericardial cavities into pericephalic mesoderm. These communicate with each other through this pericephalic coelomic space, which is now forming the craniomedian limb of the inverted U-shaped pleuropericardial cavity and may be accounted for as the essential future pericardial cavity. This cavity communicates freely with the future pleural cavity, which, in turn, passes into the peritoneal coelom. But this does not communicate with the extraembryonic coelom (fig. 8).

The reconstruction of the whole shield shows that the pleuropericardial cavity corresponds to a vague swelling presented by the ectodermal layer on the dorsal surface of the model along the lateral margins of the neural plate, and their cranial extremities are connected with each other directly beneath the the cephalic end of the head fold. Therefore, the cranial extremities of the primitive pericardial cavity and of the head fold fall practically in the same level. The caudal extremities of both lateral pericardial cavities gradually disappear at the level of the caudal termination of the neural plate. And this corresponds to the gradual diminution of the prominent ectodermic swelling on the surface of the model. In this fashion, therefore, the rhomboidal shaped head fold is surrounded by

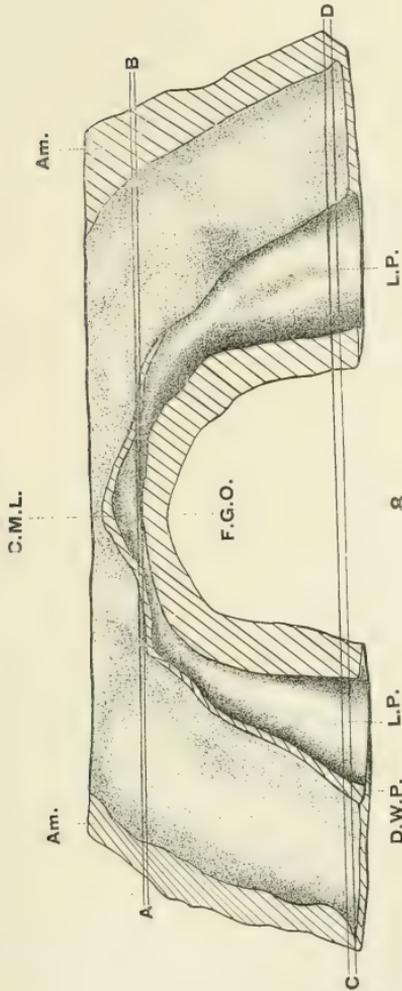


Fig. 8 Dorsal view of the reconstruction of the same embryonic shield (stage II, A), from which figures 6 and 7 were drawn. The dorsal wall of the pericardial cavity and the overhanging head fold have been removed. A-B indicates plane of section of figure 7, C-D indicates plane of section of figure 6. X 100.

the horseshoe-formed primitive pericardial cavity both laterally and cephalad.

In this stage the dimension of the craniomedian limb of the pericardial cavity is yet very narrow. Its ventrodorsal extent is not more than a lineal cleavage (fig. 7), while its craniocaudal length extends throughout five sections. In tracing the lateral limbs from the craniomedian limb, however, the pericardial cavity gradually increases in width until it reaches its maximum opposite to the hindbrain region (fig. 6), and then again a gradual reduction takes place behind this region until the coelomic cavity has completely disappeared in the region of the first mesodermic somite.

On the ventral surface of the model there can be seen the crescentic gut-groove of the entoderm at the cranial portion of the embryonic shield its apex being directed cranialward and deepened gradually until it reaches the opening of the fore-gut, which is invaginated cranialward between the ectodermal head fold and the craniomedian limb of the pericardial cavity, as a horizontal diverticulum of the yolk sac. The base of the crescentic gut-groove is directed caudalward, and gradually becomes shallower, until it has entirely disappeared at the level of the hindbrain. On both sides of the gut-groove and along the fore-gut opening there is a rounded ridge running from the latero-caudal to the craniomedian end; in both position and direction, this ridge corresponds to the horseshoe-shaped pericardial cavity.

In this stage a number of angioblasts are scattered under the considerably thickened mesoderm of the splanchnopleure throughout the full extent of the pericardial cavity. A few angioblasts can be seen in the portion of the narrowly opened craniomedian limb of the pericardial space in which the mesoderm of the splanchnopleure is in close contiguity with the underlying entoderm, and they increase in number toward the lateral limbs. In some sections in which the wide open pericardial cavity is present, endothelial tubes can be seen differentiated from the angioblasts lying under the mesoderm of the splanchnopleura, which is subsequently elevated from the entoderm and projected into the pericardial cavity as a prominent fold,

as depicted in figure 6. As the differentiation of the angioblasts into the endothelial tubes takes place irregularly, the distribution of both kinds of cells intermingles irregularly with reference to the level of sections; for example, even in the wide open pericardial region in some sections a tubular endothelium appears, while in the next succeeding section there are merely scattered angioblasts or cell strands of angioblasts. For this reason the invagination of the mesoderm of the splanchnopleura into the pericardial cavity as a prominent fold cannot be attributed simply to the development of the endothelial tubes, resulting in the increase of their volume. In this embryo, generally speaking, the angioblasts apparently predominate over the endothelium.

The dorsal aortae can be seen developed in the cephalad portion of the embryonic shield, while in many sections they are present as incompletely formed endothelial tubes resting on the entoderm on both sides of the notochordal plate (fig. 6), in their caudal extent they remain as cell strands of angioblasts.

In this embryo the foregut is present as a completely formed short entodermic tube, and the gut-groove, following caudally, is shown as a deep furrow. Not until a later stage is reached are the endothelial tubes completely developed. This corresponds apparently with the development of the human embryo, as described by Graf Spee. In the slightly younger embryonic shield the coelomic cavity in the pericephalic mesoderm is not yet formed in the median plane, where this is separated in many places by thin mesodermic bridges. But this condition is only temporary and both lateral pericardial cavities will communicate with each other, as is shown in this embryonic shield.

B. In figure 9 is represented a drawing of a midsagittal section of an embryonic shield of a guinea pig, removed from the uterus fourteen days and twenty-three hours after insemination. This series includes 172 sections, having a 7μ thickness. The figure represents a drawing of the eighty-fifth section, and this passes through the embryo just parallel and just lateral to the midaxis. As reckoned by age, this embryonic shield is just slightly older than that discussed under figures 6, 7, and 8 but,

judged by the stage of general development, the same findings can be noted. Here it is noticed that the craniomedian limb of the pericardial cavity is not more than a lineal cleavage in the pericephalic mesoderm, which extends in this plane from the more anteriorly situated extraembryonic mesoderm up to the pharyngeal membrane, where the mesoderm is entirely absent and the ectoderm and entoderm are in close contiguity.

In the peripheral two-thirds of the mesoderm in this section the cells have no definite arrangement, but are loosely and irregularly scattered. This portion can be seen as a continuation of the extraembryonic mesoderm, and the same condition is shown in figure 5B in the younger stage. In the central third of its extent the two layers of the cell band can be distinguished, namely, the mesoderm of the splanchnopleura and the somatopleura, and between them lies a craniomedian limb of the pericardial cavity. Its cranio-caudal extent is short and its cranial extremity is approximately on the same level with that of the head fold. Its sagittal long axis forms an obtuse angle with the sagittal long axis of the embryonic shield.

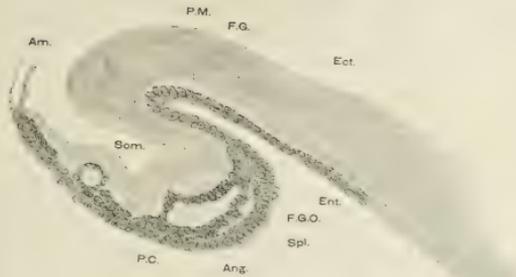
Fig. 9 The 85th section of a series of 172 sagittal sections, having a $7\ \mu$ thickness. This embryonic shield was removed from the uterus of a guinea-pig 14 days and 23 hours after insemination. The pericephalic mesoderm separates into two layers by the lineal coelem space, through which the two lateral pericardial cavities communicate with each other. The foregut has just begun to develop. $\times 150$.

Fig. 10 The 83rd section of a series of 166 sagittal sections of a $7\ \mu$ thickness of an embryonic shield of the guinea pig, removed 14 days 12 hours after insemination. The craniomedian limb of the pericardial cavity in the pericephalic mesoderm is wide open. The ventral wall of the pericardial cavity in figure 9 forms the caudal wall of it in this figure, as the reversing process occurs in the preumbilical region of the embryonic body, in conjunction with the development of the foregut, which in this embryo shows a longer lumen than in figure 9. $\times 150$.

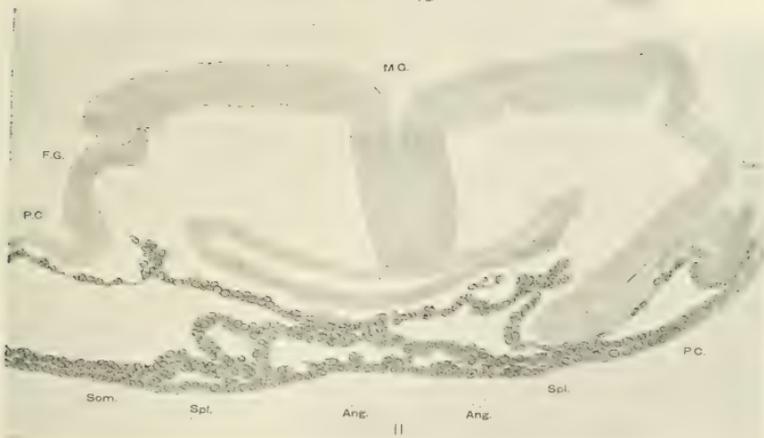
Fig. 11 The 45th section of a series of 318 cross-sections, having a $7\ \mu$ thickness of an embryonic shield of a guinea pig, removed from the uterus 15 days 14 hours after insemination. This section passes through the forebrain plate near its anterior margin. The craniomedian limb of the pericardial cavity is wide open. The splanchnopleural fold projects into the pericardial cavity, rising from the underlying entoderm. This is especially prominent on the left side of the figure. Between the splanchnopleura and entoderm a number of angioblasts are scattered. $\times 150$.



9



10



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In the younger stage the pericephalic mesoderm is situated anterior to the pharyngeal membrane, approximately in the same horizontal plane with the embryonic shield (fig. 5B). But in this stage it is brought ventrally to the pharyngeal membrane, as the foregut has begun to develop; the reversal of the pre-umbilical portion of the embryonic body accompanies this development.

C. In figure 10 is presented a drawing of a midsagittal section of an embryonic shield of a guinea pig, removed from the uterus fourteen days and twelve hours after insemination. This series includes 166 sections, having a 7μ thickness. The figure is reproduced from a drawing of the eighty third section, passing through almost exactly parallel to the midaxis of the embryonic shield. As measured by age, this embryonic shield is slightly younger than that discussed under figure 9, but the general findings of the development are slightly in advance of that of the latter.

The craniomedian limb of the pericardial cavity, which lies under the foregut, extends dorsocaudally in a cranioventral direction, and has increased its dimensions in both the ventrodorsal and craniocaudal directions (fig. 10). Compared with the foregoing embryo (fig. 9), its sagittal long axis forms an angle a little more acute with the longitudinal axis of the embryonic body. Its caudal half presents the crescentic coelom cleavage, directing its convexity caudoventralward, while its cranial half still remains as a lineal slit. The cranial extremity of the craniomedian limb of the pericardial cavity is practically situated on the same level with that of the head fold (fig. 10). It may be demonstrated, when we compare figures 9 and 10, that the backward movement of the foregut opening from the cranial extremity of the head fold is greater than the rate of progress of the head fold forward from a certain fixed point. This actual backward progress of the foregut opening brings about the lengthening of the foregut. The foregut in this embryo is longer than in the preceding embryo. Its cranial extremity is slightly caudad to that of the craniomedian limb of the pericardial cavity. The ventral wall of the foregut

coalesces with the ectoderm, indicating the pharyngeal membrane, while its dorsal wall corresponds to the cranial end of the notochord. The entoderm, which forms the dorsocaudal wall of the craniomedian limb of the pericardial cavity, is reflected from the foregut opening to the ventral wall of the craniomedian limb of the pericardial cavity, representing the so-called cardiac fold (repli cardiaque Tourneux). The cranial wall of the craniomedian limb of the pericardial cavity is formed by the ectoderm, which is reflected from the pharyngeal membrane to the proamniotic region.

A few cell strands of angioblasts can be seen between the mesoderm of the splanchnopleura and the underlying ectoderm. The splanchnopleura is present, its convexity turned caudoventrally, in accordance with the entodermic cardiac fold. Its central part has begun to invaginate into the pericardial cavity, rising from the underlying entoderm. Between these two layers the angioblasts are scattered.

Stage III

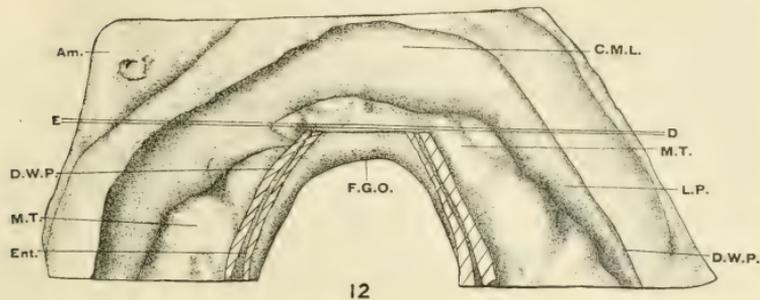
The material on which the description of stage III is based consists of two embryos, one of which was cut transversely and the other longitudinally.

A. This specimen was removed from the uterus of a guinea-pig fifteen days and fourteen hours after insemination. This series includes 318 sections, having a 7μ thickness. The plastic reconstruction of the cephalic portion of the embryo was made with wax plates, and the whole shield, reconstructed for another purpose, was used for this study.

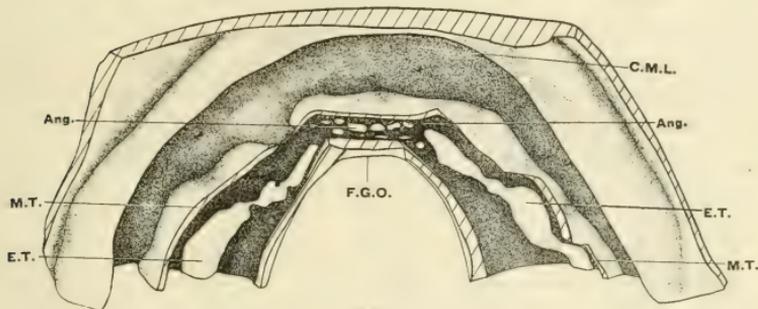
The neural groove extends from the cranial end to the caudal amnion attachment. It is wide and shallow in the caudal portion, while it is narrow and deepens toward the head fold. The head fold is divided into two primary vesicles; the cranial vesicle is wide and long, projecting laterally and cranially over the cranial and lateral walls of the cranial body elevation. The caudal vesicle is small and passes insensibly into the spinal portion. The anlagen of the trigeminal ganglia, as well as the rudiment of the otic ganglia, are to be seen. Four somites

are completely segmented, besides in their cranial and caudal territory, a somite is in process of formation. The dorsal aortae and the first aortic arch are present, while the ventral aortae are not yet completely differentiated. In the region of their anlagen the angioblasts are irregularly distributed. The foregut extends throughout twenty-two sections, appearing first in the twenty-seventh section and continuing to the forty-ninth section, while the craniomedian limb of the pericardial cavity extends throughout sixteen sections, appearing in the twenty-third section and continuing to the thirty-ninth section. The cranial end of the head fold appears in the eighth section, on account of the forward progress of the head fold. This fact can be demonstrated in the dorsal surface of the whole reconstruction model, in which the prominent swelling of the dorsal surface of the pericardial cavity is much more distinct than that of the foregoing model. The cranial extremity of the pericardial cavity disappears under the head fold slightly caudad to its cranial margin.

In this embryo the mesoderm of the splanchnopleural projects into the pericardial cavity, forming prominent folds, already presented in the previous stage, but in this stage is well developed. These folds are converted on both sides into continuous myocardial tubes. Their dorsal surfaces approach the dorsal wall of the pericardial cavity, coming nearly into contact with it. These lateral myocardial tubes are best developed at the level of the hindbrain, where they are relatively dilated and contain the well-developed endothelial tubes. On tracing cranialward, both myocardial tubes gradually diminish in height and width, until they finally disappear opposite to the foregut opening on its left side, while on the right side the myocardial tube continues into the caudad portion of the craniomedian limb of the pericardial cavity, converting it into the prominent rounded ridge of the mesoderm of the splanchnopleura. In this region the thickened mesoderm of the splanchnopleura, present as a somewhat flattened fold, is elevated above the underlying entoderm. In the space between these two layers a number of angioblasts can be seen (fig. 11 and 12). In tracing still farther cranialward, the relatively thin layer of mesoderm



12



13

Fig. 12 Dorsal view of the reconstruction of the same embryonic shield (stage III, A) from which figure 11 was drawn. Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. At the caudal part of the craniomedian limb of the pericardial cavity the splanchnopleura projects into the pericardial cavity, forming the prominent fold, which is absent in front of the cranial extremity of the myocardial tube on the left side. *E-D* indicates plane of section of figure 11. $\times 100$.

Fig. 13 Dorsal view of the reconstruction of the same embryonic shield (stage III, A) from which figures 11 and 12 were drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to show the underlying endothelial tubes and angioblasts. Angioblasts are scattered under the splanchnopleural fold at the caudal part of the craniomedian limb of the pericardial cavity, almost connecting both cranial extremities of the lateral endothelial tubes. But angioblasts are absent in front of the cranial extremity of the lateral endothelial tube on the left side, where the splanchnopleura has not risen from the underlying entoderm. $\times 100$.

of the splanchnopleura remains attached to the underlying entoderm; between them no angioblasts can be seen (fig. 13).

It can be ascertained that the formation of the mesodermal splanchnopleural folds occurs *in loco* and progresses cranialward, until both cranial extremities of the myocardial tubes will ultimately unite and communicate with each other at the craniomedian limb of the pericardial cavity. The craniomedian limb of the pericardial cavity increases its dimensions both in the ventrodorsal and in the craniocaudal directions, while the formation of the myocardial anlage in this portion remains in its primitive condition. In the hindbrain region the pericardial cavity reaches its maximum width in proportion to the myocardial and endothelial development. But it shows here a rather narrower space in the ventrodorsal direction, on account of the dorsal expansion of the myocardial tubes. In tracing still further caudalward, the pericardial cavity gradually narrows until it entirely disappears in the somitic region, parallel with the gradually diminishing splanchnopleural folds and endothelial tubes.

The endothelial tubes are differentiated at great length, extending throughout nearly the whole extent of the lateral pericardial cavity. But in many places these tubes are irregularly interrupted, their continuity bridged by angioblast cords. The endothelial tubes terminate cranially opposite to the foregut opening on the right side and slightly caudad to it on the left side. In front of these terminations a number of angioblasts are scattered. Extending still farther craniomedially, by means of these angioblasts, the cranial extremities of the endothelial tubes are connected with each other through the middle plane underneath the flat splanchnopleural folds of the craniomedian limb of the pericardial cavity (fig. 13). There is a distinct significance in the fact that these angioblasts are directly derived from the mesoderm of the splanchnopleural cells *in loco*. Moreover, in some other parts the angioblasts or endothelial cells are undoubtedly connected with the thickened and indented mesoderm of the splanchnopleura. Therefore, it is conceivable that the productive activity of these cells from the mesoderm of the splanchnopleura is still continued in this embryo.

Stage IV

The material on which the following description of stage IV is based consists of two embryos, one of which was cut transversely and the other longitudinally.

A. This specimen was removed from the uterus of a guinea-pig fourteen days and eight hours after insemination. This series includes 566 sections, having a 5μ thickness, from the cranial margin of the head fold to the caudal end of the mesodermic thickening of the allantois. The plastic reconstruction of the cephalic portion of the embryonic body was made with wax plates.

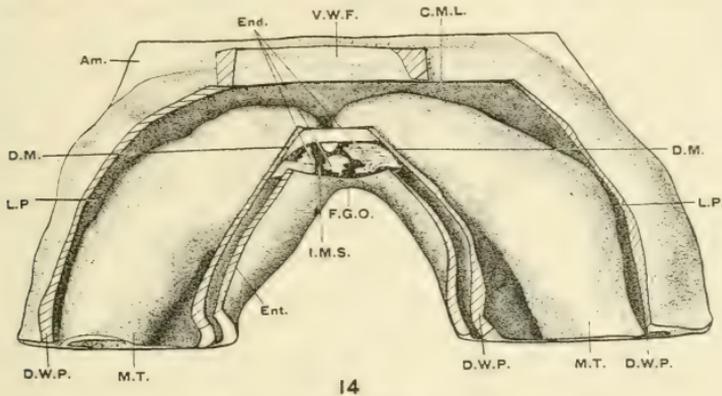
The head fold has progressed cranial- and dorsalward. Its cephalic extremity is represented in that of the embryonic shield. There are present seven pairs of mesodermic somites, the first and last being small and indistinctly segmented. The neural groove extends from the cranial end to the caudal amnion attachment. In the hindbrain region the neural groove shows very narrow and deep as both the neural plates approach each other.

In the model it can be recognized that the craniomedian limb of the pericardial cavity increases its dimensions in the ventrodorsal direction, while its lateral and craniocaudal extent remains approximately unchanged in comparison with the previous stage III. The cranial extremity of this portion extends at its dorsal part into the mesodermic cavity of the mandibular region on both sides. While their outline gradually approaches the horizontal plane, the caudal extremity of this portion is continued into both the lateral pericardial cavities, which gradually diminish in width caudalward. In the region of the second somite they entirely disappear.

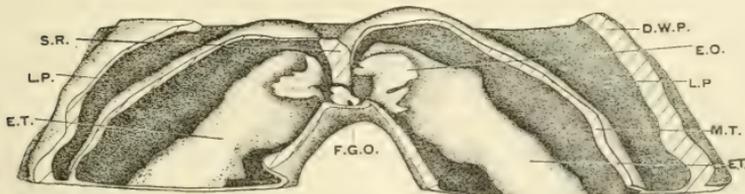
The formation of both the lateral myocardial tubes, which has been discussed in stage III, are considerably developed and have so far progressed cranialward, that their cranial portions have partially come into contact and been fused together. Through this portion the myocardial tubes communicate with each other. On the dorsal surface of this fused portion of the

lateral myocardial tubes the myocardial walls are reflected directly onto the dorsal wall of the pericardium, thus forming the dorsal mesocardium on both sides. Between the lateral mesocardial layers there is present an irregular triangular space, which we purpose to designate as the intermesocardial space and through which the endothelial offshoots come out from the myocardial cavity onto the space between the mesocardial layers and the floor of the foregut. Its apex is directed cranialward, where the lateral mesocardial layers come in contact, marking the cranial margin of the communicating myocardial cavity. Its basal portion is directed caudalward and corresponds to the foregut opening, by which the lateral mesocardium layers diverge from each other and continue farther caudalward along the lateral myocardial tubes (fig. 14). Between the above-mentioned adherent cranial margin of the mesocardium and the foregut opening, the lateral myocardial cavities communicate with each other across the middle plane to the extent of eight sections. From this communicating myocardial cavity are sent out two short cranial diverticula on either side, separated by a septal wall in the middle plane, corresponding to the cranial extremities of the lateral myocardial tubes. These diverticula are present as the rounded myocardial horns, directed cranialward and separated from each other by their own inner walls. These inner walls are caudally converted into a wedge-shaped prominent ridge, which continues into the communicating portion of the myocardial cavity and gradually diminishes caudalward (fig. 15). The communicating portions of the lateral myocardial tubes are directly continued into the lateral myocardial tubes caudolaterally on both sides and they are separated from each other by the foregut opening. The ventral wall of the communicating portion of the myocardial cavity is reflected onto the ventral wall of the pericardium and is recognized only in the caudal portion. The reflection points from the myocardium to the ventral pericardial wall are fused together at the cranial part, but at the caudal part the reflection points diverge from each other and a triangular space remains between them in just the same manner as can be seen in the dorsal wall.

This ventral triangular space is covered by the entoderm cephalad to the foregut opening, while the dorsal intermesocardial space is covered by the foregut floor. Ventrally to the communicating myocardial cavity, the pericardial cavity passes from side to side, because of the absence of the ventral mesocardium. Along the ventral mesodermic reflection the anlage of the septum transversum of His will be presented in the future development, and the mesodermic reflection may be erroneously taken for



14



15

Fig. 14. Dorsal view of the reconstruction of an embryonic shield (stage IV). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. The two lateral myocardial tubes are partially confluent, slightly cephalad to the foregut opening. The two short cranial horns of the myocardial tubes are directed toward the top of the page. $\times 100$.

Fig. 15. Dorsal view of the reconstruction of the same embryonic shield (stage IV) from which figure 14 was drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to expose the underlying endothelial tubes. The two lateral endothelial tubes approach most closely to each other at the confluent myocardial portion, where independent endothelial cells are interposed between the two tubes. $\times 100$.

the ventral mesocardium, if a single section of this portion should be examined, as many workers claim the existence of the ventral mesocardium in mammals.

The endothelial tubes are well developed and are enclosed within the myocardial cavities on both sides. Their lumina are patent throughout their cranial extent, their cranial extremities terminate blindly opposite to the cranial extremities of the myocardial tubes, where the myocardial tubes are projected into the pericardial cavity, like the rounded lateral horns on either side, which contain the cranial extremities of the myocardial cavity. In tracing caudally, the endothelial tubes reduce their calibers gradually and they are irregularly interrupted in their continuity by angioblasts. They entirely disappear in the somitic portion, where the lateral pericardial cavities assume a narrow and horizontal space and the splanchnopleural folds have entirely disappeared. The lateral endothelial tubes are most remarkably dilated and considerably approximated to each other at the communicating myocardial cavity, where the independent intermediate endothelial cells can be seen between the endothelial tubes. Throughout many sections in the communicating myocardial cavity, the endothelial tubes give off their endothelial offshoots from their dorsomedian aspects, coursing dorsolateralward, between the dorsal mesocardium and the floor of the foregut. These offshoots may be considered the future truncus arteriosus.

Owing to the gradual transition from these dilated endothelial tubes into the portion of the vitelline veins caudalward, their demarcation cannot be pointed out on the endothelial tubes nor on the myocardial tubes.

The dorsal aortae and the first aortic arch are completely developed, while the ventral aortae are incompletely differentiated. In their anlagen a number of angioblasts are distributed irregularly.

Stage V

The material on which the following description of stage V is based consists of two embryonic shields, which were cut trans-

versely. The partial plastic reconstruction of the cephalic portion and the reconstruction of the whole embryonic shield, made for another purpose, were used for this study.

A. This specimen was removed from the uterus of a guinea pig fourteen days and eight hours after insemination. The series includes 612 sections, having a 5μ thickness. As measured by age, this embryonic shield is slightly younger than that discussed in stage IV. As judged by the stage of general development, it is slightly more advanced, indicated by the facts that eight somites are present and that the medullary groove is much deeper and narrower in the hindbrain region, so that to a great extent both neural plates are in contact; here it passes insensibly into the spinal region. The forebrain plate still remains wide open, projecting cranially and laterally over the cranial and lateral wall of the anterior body elevation. It is, moreover, bent considerably ventralward. In the model it can easily be recognized that, owing to the fact that this embryonic shield is considerably folded off from the yolk sac, it is in general thicker in the ventrodorsal diameter and narrower in the lateral diameter than that in stage IV. In accordance therewith, the cranial extremities of the lateral myocardial tubes are forming a more acute angle than that of the previous stage. The craniomedian limb of the pericardial cavity increases its ventrodorsal and craniocaudal dimensions, while its lateral diameter diminishes on comparison with the embryo of stage IV, in proportion to the rounded outline of this embryo. The craniomedian limb of the pericardial cavity is elongated at its dorsal part into the mandibular mesoderm. In coursing caudalward, the lateral pericardial cavities gradually diminish their width, and at the same time their outline approaches the horizontal plane as a whole. They disappear entirely opposite to the fourth somite. The direction of the lateral myocardial tubes tends to their running parallel to each other. The lateral myocardial tubes are considerably dilated at their cranial portion, where they abruptly become voluminous in comparison with their caudal portion. The transitional points of these two different portions are situated a little caudad to the foregut opening on both sides,

where the myocardial tubes mark slight indentations. These indentations indicate the future atrioventricular constriction (fig. 16).

The cranial extremities of the lateral myocardial tubes become more voluminous as compared with those of the previous stage and are projected cephalad into the pericardial cavity as large lateral rounded horns. They are separated from each other by their own inner walls, which fuse caudally into one septal wall and, as we trace still farther caudally, we find them converted into the prominent wedge-shaped ridge which projects into the communicating myocardial cavity. This ridge gradually diminishes in height caudalward until it has entirely disappeared in the middle of the communicating cavity (fig. 17).

The dorsal wall of the fused myocardial tubes is reflected directly onto the dorsal wall of the pericardium, forming the dorsal mesocardium on both sides. These lateral mesocardial layers come to fusion in a region a little cephalad to the foregut opening, where it makes the cranial margin of the communicating myocardial cavity. On the dorsal surface of the fused myocardial portion an irregular intermesocardial space can be seen, covered by the floor of the foregut. Its apex is directed cephalad, corresponding to the point where the lateral mesocardium layers are fused together. Its base is directed caudad, corresponding to the foregut opening. Its sides are formed by the lateral mesocardial layers. Corresponding to this intermesocardial space, the lateral myocardial tubes communicate with each other through the median plane throughout the extent of nine sections. In a similar way, the ventral wall of the fused myocardial tube is reflected onto the ventral wall of the pericardium, but only in its caudal portion. Between these two lines of the mesodermal reflection there remains a narrow space free from the mesoderm and covered directly by the entoderm. However, these lines of reflection on the ventral wall are disposed in a rather transverse direction and are located only for a short extent in the caudal part of the communicating myocardial tube, while on the dorsal surface the lines of reflection of the mesocardium are directed rather longitudinally and extend

throughout the whole length of the communicating myocardial tube. The communicating portion of the myocardial cavity terminates blindly in the cranial diverticulum cephalad, cor-

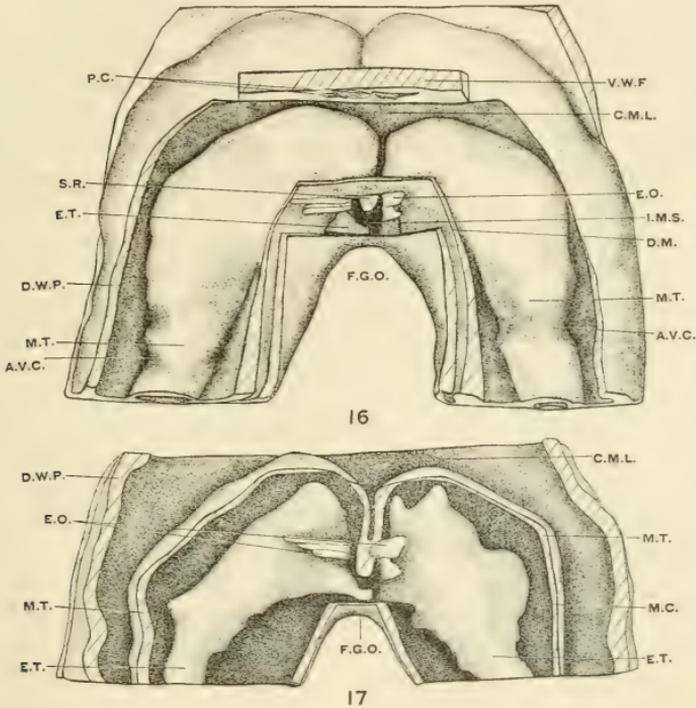


Fig. 16 Dorsal view of the reconstruction of an embryonic shield (stage V). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. The two lateral myocardial tubes become quite voluminous, especially at their cranial portions, which continue farther caudalward, gradually diminishing in size. The atrioventricular constriction is marked on the surface of the myocardial tubes, caudad to the foregut opening on both sides. The two cranial horns of the myocardial tubes become enlarged, and they are directed toward the top of the page. $\times 100$.

Fig. 17 Dorsal view of the reconstruction of the same embryonic shield (stage V) from which figure 16 was drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to expose the underlying endothelial tubes, which are apparently enlarged at their cranial portions, where they most nearly approach each other. $\times 100$.

responding to the cranial extremities of the lateral myocardial tubes, while caudally it is elongated into the lateral myocardial tubes, which are diverged by the foregut opening, and their lumina are gradually diminished toward the somitic region.

In general, the endothelial tubes are much more developed than those of the previous stage, since they have become deeper and wider. At the widest portion of the endothelial tubes, corresponding to the communicating myocardial cavity, the endothelial tubes approach each other so that they come nearly into contact. On these portions, throughout many sections, the endothelial tubes give off a number of endothelial offshoots from their dorsomedian surface into the space between the dorsal mesocardium and the foregut floor. From these portions they become gradually narrower, toward both the cranial and caudal directions. The cranial extremities of the endothelial tubes terminate blindly opposite to the cranial myocardial extremities, while caudally they continue into the portion of the vitelline veins. The endothelial tubes assume the distinctly narrow calibers opposite to the atrioventricular constriction. The endothelial tubes sprout out into innumerable tenuous fibrils, often forming a feltwork, which occupies the wide space between the myocardium and the endothelium.

The dorsal aortae and the first aortic arch are developed, while the ventral aortae are not completely formed, as in their anlagen a number of angioblasts are scattered.

Stage VI

The material on which the following description of stage VI is based consists of one embryo, cut transversely. The plastic reconstruction of the cephalic portion of the embryo was made with wax plates.

This specimen was removed from the uterus of a guinea pig fourteen days and eight hours after insemination. The series includes 582 sections, having a 5μ thickness. As measured by age, this embryonic shield is the same as that of the previous embryo. As reckoned by general development and special de-

velopment of the heart, it is considerably advanced over the preceding embryo. Eight well-segmented somites are present. The medullary groove, extending from the cranial end to the caudal amnion attachment, is as deep and narrow as in the previous embryo. The form of the embryonic shield is, in general, more rounded in comparison than with the foregoing embryo, as the ventrodorsal diameter of this embryo is apparently increased while its lateral diameter has remained unchanged. The first visceral pouch and the oral pit are developed; in these places the entoderm coalesces intimately with the ectoderm.

The reconstruction shows that at this stage of development the craniomedian limb of the pericardial cavity increases considerably in the craniocaudal dimension and in the ventrodorsal dimension. The craniomedian limb of the pericardial cavity communicates caudally with the lateral pericardial cavities. On coursing caudally, these become gradually narrower, until they disappear entirely opposite to the sixth somite. The craniomedian limb of the pericardial cavity is elongated cranially at its dorsal part into the mandibular portion, lying under the foregut floor. The caudal half of the ventral surface of the craniomedian limb of the pericardial cavity is covered by the yolk sac, while its cranial half and all other surfaces are covered by the amnion.

In this stage the fused portion of the lateral myocardial tubes increases remarkably throughout its craniocaudal extent. In accordance therewith, the cranial bilateral myocardial horns, which correspond to the cranial extremities of the lateral myocardial tubes, and predominate in the craniomedian limb of the pericardial cavity in the previous stage, apparently diminish their dimensions in this embryo, and show only their rudiments. They assume only short and wide bilateral processes, divided by a shallow and wide intervening groove. In the previous stage this groove was present as a narrow and deep sulcus. Subsequently, the inner walls of these horns diverged markedly from each other (fig. 18). The wedge-shaped ridge which, in the previous stage, projected into the communicating myocardial cavity at its middle cranial wall, as a caudal continuation

of the converted septum walls of the cranial bilateral myocardial horns, is considerably retired cranialward in this embryo. Therefore, the cranial wall of the communicating myocardial cavity approaches in such a manner toward the cranial wall of the pericardium as to come nearly into contact with it and, simultaneously, the communicating myocardial cavity is elongated cranialward. The fused portion of the myocardial tube

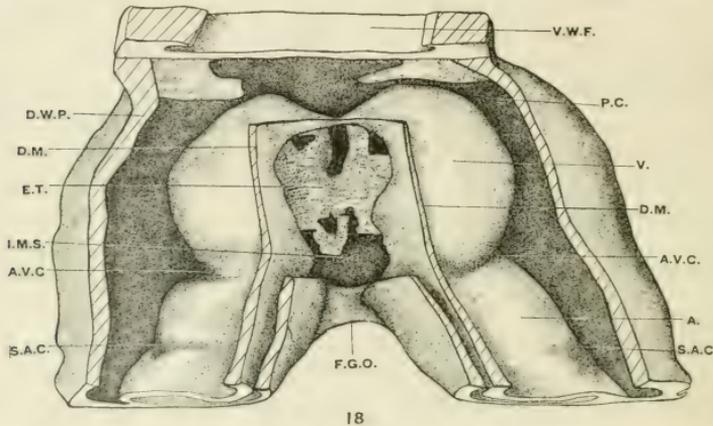


Fig. 18 Dorsal view of the reconstruction of an embryonic shield (stage VI). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. The confluent portion of the two lateral myocardial tubes is considerably elongated in the craniocaudal direction. The two cranial horns of the myocardial tubes diminish to short rudiments, as their septal wall retires cranialward. They are directed toward the top of the page. $\times 100$.

becomes distinctly narrower and thicker in comparison with that of the previous stage. In two of the same magnified models ($\times 300$) the widest lateral diameter of this portion is calculated as 14.5 cm. in this embryo, instead of 19 cm. of the previous embryo, while the ventrodorsal diameter of this portion presents 5.1 cm. in this embryo and 3 cm. in the former embryo.

The fused myocardial tube is reflected directly onto the dorsal wall of the pericardium, and thus forms the dorsal mesocardium on both sides. Between the lateral mesocardial layers there can be seen a long rectangular intermesocardial space; its

plane is approximately parallel with the horizontal. Its cranial margin is formed by the fused portion of the mesocardial layers in the middle line; its caudal margin corresponds to the foregut opening, while both lateral margins are represented by the lateral mesocardial layers, which continue farther caudalward, diverted by the foregut opening. In accordance with this mesocardial space, both myocardial tubes communicate freely with each other through the median plane, and thus form

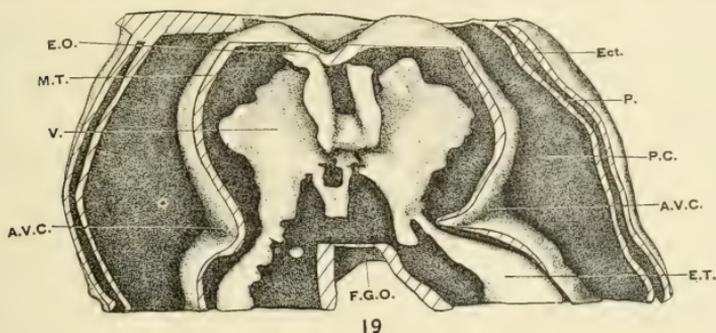


Fig. 19 Dorsal view of the reconstruction of the same embryonic shield (stage VI) from which figure 18 was drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to expose the endothelial tubes. The two lateral endothelial tubes have fused and communicate with each other at a middle third of the ventricle, where they most closely approach each other in figure 17. The lateral endothelial tubes apparently diminish their size opposite to the atrioventricular constriction. $\times 100$.

the craniomedian limb of the myocardial cavity. This limb of the myocardial cavity is bifurcated caudally into the lateral myocardial tubes, which are diverged from each other by the foregut opening and in which the vitelline veins are enclosed, leading cranially into the craniomedian limb of the myocardial cavity (fig. 19). In brief, the myocardial anlage presents cranially two short rudimentary horns, which terminate blindly as the cranial myocardial extremities, while caudally there are two lateral myocardial prolongations, into which the vitelline veins enter. Between these four extremities the myocardial wall is relatively considerably expanded dorsal, lateral, ventralward and

contains the widest portions of the endothelial tubes, which are united in the communicating cavity. This region corresponds to the future ventricle region. On the midsagittal line of the ventral surface of this fused myocardial portion a shallow longitudinal groove can be seen.

On the ventral aspect of the fused myocardial portion the ventral myocardial layer is reflected onto the ventral wall of the pericardium, but this is limited to a short length, extending only to the caudal part of this portion.

The transition from the cranial expanding ventricle to the caudal myocardial prolongations is indicated by an annular constriction, which is produced by the infolding of the whole myocardial wall, a little deeper on the right side than on the left. This indicates the atrioventricular constriction and is situated at the level slightly cephalad to the foregut opening on both sides.

Proceeding caudally from this constriction, the lateral myocardial tubes reduce their calibers abruptly and diverge from each other. On the surface of these caudal prolongations of the myocardial tubes there are present indefinite, shallow indentations at the level slightly caudad to the foregut opening, and these constrictions have been regarded as the future sinoatrial construction.

The lateral endothelial tubes are partially fused and their lumina communicate with each other, for their inner walls have been absorbed throughout seven sections. This portion is situated in the middle third of the bulging ventricle anlage, where in the previous stage both endothelial tubes were closely approximated and presented the greatest dilation and where in this embryo also the endothelial tube is greatly expanded.

From this fused portion of the endothelial tube the two cranial horns and two caudal prolongations are given off. The bilateral cranial horns are short and gradually diminish in size cranialward, until they terminate in a pointed apex, opposite to the cranial extremities of the myocardial horns. From the dorsomedian part of these cranial endothelial horns a number of endothelial branches are given off. These endothelial

branches are connected with the ventral aortae through the intermesocardial space.

Bilateral caudal prolongations are given off on both sides from the caudal aspect of the fused endothelial tube. Continuing caudalward, both endothelial tubes gradually diminish their calibers, until the lumina have entirely disappeared at the atrioventricular constriction. Still further caudalward from this constriction, again they begin to dilate their calibers gradually and continue into the endothelial vitelline veins without any indication at their transitional point. At the atrioventricular constriction the endothelial tubes closely approach the infolding of the myocardial wall, while in the ventricle the intervening space between the myocardium and endothelium is relatively wide.

From the caudal aspect of the fused endothelial tube another intermediate endothelial branch is given off caudally. This branch is situated between the endothelial prolongations and terminates at the atrioventricular constriction.

The dorsal aortae and the first aortic arch are developed, while, in many sections, the ventral aortae are interrupted by angioblasts.

Stage VII

The material on which the following description of stage VII is based consists of one embryonic shield, which is cut transversely. The plastic reconstruction of the cephalic portion of the embryo was made from wax plates.

The specimen was removed from the uterus of a guinea-pig fourteen days and eleven hours after insemination. The series includes 418 sections, having a 7μ thickness, from the cephalic end of the head fold to the end of the mesodermic thickening of the allantois. Nine pairs of well-segmented somites were found, each somite showing a thick wall and enclosing a uniform cavity with a compact arrangement of cells, except two caudal somites, which contained no cavity nor presented the regular arrangement of the cells.

The medullary canal is closed from the second somite to the last, but elsewhere the medullary plates remain open. The notochordal plate is separated from the entoderm throughout from the second somite to the last, but elsewhere it is still connected with the entoderm. The first and second visceral pouches are developed, in which the ectoderm and entoderm have tightly coalesced. The oral pit is formed and the pharyngeal membrane becomes quite thin.

In this embryo the pericardial cavity is closed in all directions, forming a sac, except at the dorsal part of its caudal extremities, where the pleuropericardial passages are opened on each side. These passages are represented by the narrow coelomic space, which continues farther caudalward into the peritoneal cavity and they are situated dorsomedian to the myocardial coat of the vitelline veins. From the dorsal part of the cranial extremity of the pericardial cavity a slit-like space is elongated into the mandibular region.

The surface of the pericardium is covered by the yolk sac ventrally, while laterally, cranially, and dorsally it is covered by the amnion. The pericardial cavity shows a wide space around the myocardium. Between the cranial extremity of the myocardium and the cranial wall of the pericardium there remains a wider interval than that of the embryo of stage VI.

The myocardium presents cranially an undivided cranial extremity, which expands considerably in all directions and assumes a sac form, while caudally this myocardial sac is bifurcated into two rather slender myocardial prolongations, in which the endothelial vitelline veins are enclosed on both sides. The transition from the cranial myocardial sac to the bilateral myocardial tubes is indicated by the deep atrioventricular constriction, at the level slightly cephalad to the foregut opening. This constriction is produced by the infolding of the whole myocardial wall and shows apparently deeper on the right side than on the left. On the ventral surface of the ventricle there can be seen a shallow groove in the midsagittal line at its caudal half, and in accordance therewith the whole thickness of the myocardial wall is slightly infolded into the myocardial cavity.

This superficial groove and the infolding of the myocardial wall are located in the caudal half of the ventricle, for they gradually disappear toward its cranial extremity, which is of conical form. The ventral myocardial layer is reflected onto the ventral wall of the pericardium, but is confined to the atrial region to a short length opposite to the foregut opening.

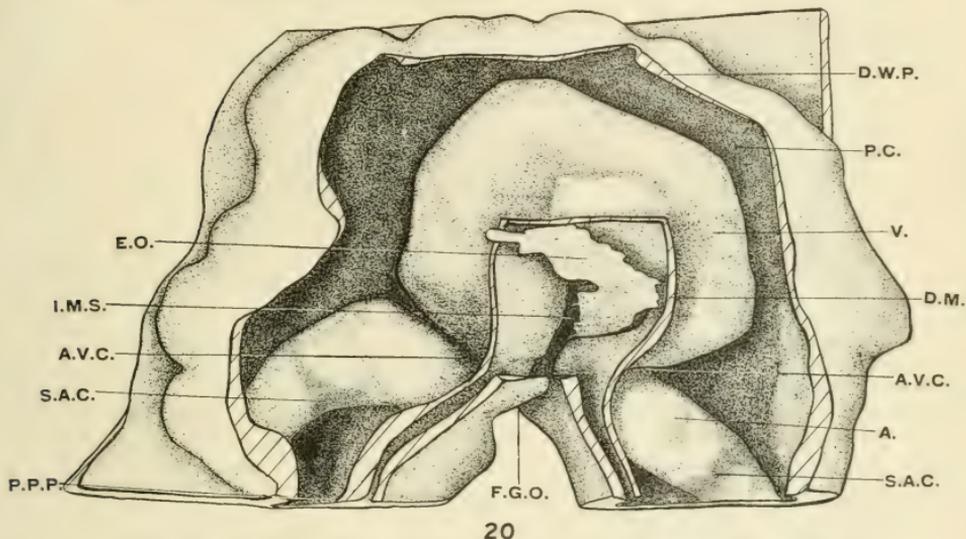


Fig. 20 Dorsal view of the reconstruction of an embryonic shield (stage VII). Dorsal wall of the pericardium has been removed to show the pericardial cavity and the myocardial tubes. The myocardium shows cranially a single sac-formed ventricle, which bifurcates into two slender myocardial tubes caudalward. The transition between them is marked by the atrioventricular constriction cephalad to the foregut opening. A single cranial extremity of the ventricle is directed toward the top of the page. $\times 100$.

On the dorsal aspect the myocardial wall is reflected onto the dorsal wall of the pericardium and forms the dorsal mesocardium on both sides. The cranial extremity of the dorsal mesocardial attachment corresponds to a middle third of the ventricle, and from this point it continues farther caudalward. Consequently, the cranial half of the ventricle is free from the mesocardium (fig. 20). Between the lateral mesocardial layers

there can be seen an irregular triangular intermesocardial space. Its plane is directed caudodorsalward on account of the abrupt dorsal expansion of the ventricle. Its apex is, therefore, situated caudoventrally and is formed by the lateral mesocardial layers, approaching contact, opposite to the atrioventricular constriction, while its basal portion is directed craniodorsally and corresponds to the portion where the lateral mesocardial layers come to fusion and mark their cranial extremities.

On the caudal surface of the ventricle the right half assumes an apparently wider dimension than the left. This is attributed partly to the exceeding expansion of the myocardial wall in the laterocaudal direction on the right half and partly to the deeper infolding of the atrioventricular constriction on the right side. On this bulging portion of the caudal extremity of the ventricle at the right half the caudal extremity of the right ventricle will be developed, and this is shown distinctly in the next stage.

The lateral myocardial tubes of the atrium are diverged from each other by the foregut opening. The right atrium is practically beginning just opposite to the foregut opening, while the left one begins slightly cephalad to it, for in comparison with the right side, the left atrioventricular constriction is shallower and situated slightly cephalad.

On the myocardial tubes of the atrial portion indefinite indentations, indicated as the sino-atrial constriction, can be recognized. These are between the atrioventricular constriction and the level of the pleuropericardial passages. This is especially noticeable on the left side.

The lateral endothelial tubes are fused together throughout the cranial two-thirds of the ventricle. Its cranial extremity terminates as a single conical apex opposite to the cranial extremity of the myocardial ventricle. At this fused portion the endothelial cavities communicate with each other and show considerable dilation. In tracing caudalward from this united portion, the tubes are separated from each other, even though they appear to approach each other. At the atrioventricular constriction the endothelial tubes present their smallest size and

simultaneously they approach closely to each other. Proceeding still farther caudally from this portion, they are diverged from each other by the foregut opening and again assume a gradual enlargement of their calibers. At the caudal part of the ventricle, where the endothelial tubes are separated, they present an asymmetrical size, for the right one is extraordinarily de-

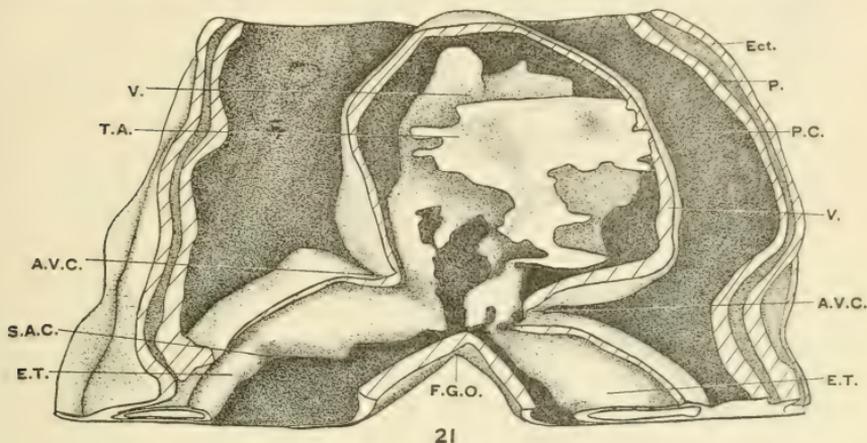


Fig. 21 Dorsal view of the reconstruction of the same embryonic shield (stage VII) from which figure 20 was drawn. Dorsal walls of the pericardium and of the myocardium have been removed to expose the endothelial tubes. The two lateral endothelial tubes have fused and communicate with each other throughout the cranial two-thirds of the ventricle. The ventricular endothelial tubes show a distinct asymmetry, due to the extraordinary enlargement of the right side, regardless of the fused or non-fused portion. The endothelial tube is elongated dorsalward from the dorsal surface of the enlarged right endothelial tube, passing through the intermesocardial space. This endothelial elongation is bifurcated into the two lateral branches, which are continuous into the ventral aortae. $\times 100$.

veloped and expended in the lateral and caudal directions, forming a curvature whose convexity is turned laterocaudalward. At this endothelial portion the endothelial tube is elongated vertically dorsalward and comes out from the myocardial cavity onto the foregut floor through the intermesocardial space. The cranial part of this endothelial elongation is bifurcated into two later branches which connect it cranially with the corresponding

ventral aortae. The asymmetrical development of the endothelial ventricle corresponds to the myocardial asymmetry in the ventricle, which has been mentioned above. In this part of the ventricle the most important change will be noted in the next stage, here developing, namely, the right limb of the ventricle. And this change is initiated in this embryo as a considerable asymmetrical expansion of the caudal extremity of the ventricle on the right side.

At the ventricle the endothelial tubes are separated from the myocardial wall by a wide intervening space, but they gradually approach each other in the direction of the atrio-ventricular constriction caudalward, as in the caudal part of the atrium no more intervening space can be pointed out between the myocardial wall and the endothelium (fig. 21).

The transition from the atrial endothelial tubes into the sinus portions can be pointed out by the abrupt decrease of the endothelial caliber on the left side, corresponding to the relatively distinct sino-atrial constriction of the myocardium. But on the right side the atrial endothelial tube continues farther caudalward without any demarcation, in accordance with the relatively indistinct myocardial constriction.

On both sides the ductus cuvieri can be seen opening into the sinus venosus. The dorsal aortae and first aortic arch are well developed, while in many places the ventral aortae still retain the plexus form.

Stage VIII

The material on which the following description of stage VIII is based consists of one embryonic shield, which is cut transversely. The plastic reconstruction of the cephalic portion of the embryo was made; the reconstruction of the whole embryonic shield, made for another purpose, was used for this study.

This embryo was removed from the uterus of a guinea pig fourteen days and twelve hours after insemination. The series includes 408 sections, having a 10μ thickness, from the cephalic end of the head fold to near the caudal extremity of the allantoic mesodermic thickening. Nine pairs of the well-seg-

mented somites are present and the tenth is in process of formation; each somite shows the thick wall and encloses a uniform cavity. The neural canal is closed from the region of the hindbrain to the region of the last somite, though in the fore- and midbrain region it still remains open. The cranial flexure is shown in the region of the midbrain and that of the forebrain is bent downward and forward, bringing it to a plane parallel with the long axis of the hindbrain. The foregut is closed to the first somitic region. The first visceral pouch is found in the region of the midbrain with the entoderm and ectoderm coalesced, while the second visceral pouch is in process of formation also in the region of the hindbrain, in which region between the entoderm and ectoderm a thinner layer of the mesoderm than elsewhere is found interposes. The oral pit is well formed, the pharyngeal membrane is present as a thin single layer of cells. On the ventral surface of the model the edge of the foregut opening is elevated by two prominent limbs, which on each side are confluent into an extensive ventral bulging cranially to the foregut opening. In position and direction this corresponds to the pericardial cavity, containing the voluminous heart.

The pericardial sac is closed except at the dorsomedian part of its caudal extremity, where the pleuropericardial passages are found. In the region of the sinus venosus each one of the bilateral pericardial cavities is divided into a median and a lateral part by the myocardial fold and in the region of the pleuropericardial passages the lateral parts of the bilateral pericardial cavities terminate blindly caudalward, so that only their median portions are continued caudally into the peritoneal cavity. Accordingly, these passages are represented merely by narrow, crescentic coelomic spaces, dorsomedian to the vitelline veins, proceeding caudally and mesially, crossing with the vitelline veins, which run cranially and mesially.

On account of the considerable enlargement of the muscular heart, the pericardial space is, in general, proportionately reduced, especially in the well-developed ventricular portion a simple narrow space surrounds the muscular sac of the ventricle.

In the region of the atria and the sinus venosus a relatively wide space intervenes between the rather flat muscular tubes and the pericardial wall (fig. 22). There are present two distinct constrictions on the tubular muscular heart, infolding the whole thickness of the myocardial wall, one of which represents the atrioventricular constriction and the other the sino-atrial constriction.

The atrioventricular constriction is present asymmetrically on both sides; on the right side it is marked more deeply and situated slightly caudad, while on the left side it is shallower and lies a little cephalad. Therefore, this constriction forms an oblique angle with the long axis of the muscular heart.

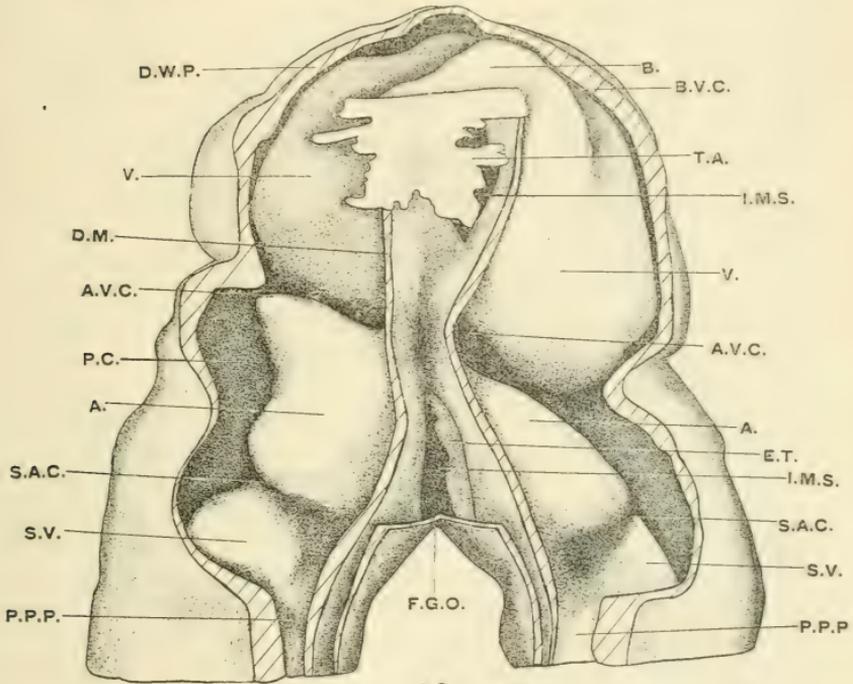
On the contrary, the sino-atrial constriction is marked more deeply on the left side and is situated slightly cephalad to the foregut opening, while on the right side it is less deeply constricted and is situated slightly caudad, just opposite to the foregut opening.

The ventricle can be divided into two lateral limbs by a ventral and a dorsal longitudinal sulcus. On the dorsal surface it is marked along the attachment line of the dorsal mesocardium and terminates caudally opposite to the right margin of the atrioventricular constriction, while its cranial extremity gradually disappears at the portion where the bulbus cordis is differentiated from the dorsal wall of the ventricle. On the ventral surface the longitudinal sulcus extends to a caudal third of the ventricle.

At the caudal part of the ventricle, for a short length, both lateral limbs are divided into two completely independent cavities by the septal wall. The caudal extremity of the right ventricle is shown as the conical process, projecting caudolaterally and terminating blindly, while at the caudal extremity of the left ventricle the atrioventricular canal opens, which is formed by the infolding of the muscular wall, corresponding to the atrioventricular constriction.

The septal wall between the two lateral limbs at the caudal part of the ventricle is farther continued cranialward and is converted into wedge-shaped prominent ridges at the inner sur-

face of the ventral and dorsal wall of the ventricle, in relation with longitudinal sulci on the external surface. These ridges have gradually disappeared within a caudal third of the ventricle (fig. 23). These prominent ridges show their anlage only



22

Fig. 22 Dorsal view of the reconstruction of an embryonic shield (stage VIII). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardium, which is subdivided into several individual portions (bulbus cordis, ventricle, atrium, sinus venosus, etc.) by the distinct bulbo-ventricular atrioventricular, sino-atrial constrictions. $\times 100$.

on the ventral wall of the ventricle, near its caudal end, as seen in the previous stage. Consequently, there can be but little doubt that these folds cannot be regarded as the remnants of the primitive cardiac septum.

The bulbus cordis is differentiated from the dorsal wall of the right ventricle near its cranial end, bulging out its wall cranial-dorsal and laterally. Its ventral wall is distinctly separated from the dorsal wall of the right ventricle at its cranial portion, projecting cranialward as an independent muscular sac, while in its caudal portion there can be noted no distinct demarcation between the wall of the bulbus cordis and that of the right ventricle. At the left and cranial sides of the bulboventricular junction, a deep external furrow can be seen, accompanied by a consequent infolding of the muscular wall. On the right side the bulboventricular furrow is indefinitely marked only its cranial part, while in its caudal part it has disappeared entirely and insensibly continues into the dorsal wall of the right ventricle. On this account the bend of the heart tube at the bulboventricular junction is effected toward the right side, turning its concavity to the left side, beneath the left layer of the dorsal mesocardium.

On the dorsal surface of the bulbus cordis there can be seen a triangular intermesocardial space, its plane is directed dorsally and slightly to the left. Its apex is directed caudally and at a lower level, where the demarcation between the bulbus cordis and ventricle wall show indefinitely. Its base is situated cranially and at a higher level, where the wall of the bulbus cordis is distinctly demarcated from that of the ventricle, and there marks the cranial termination of the dorsal mesocardium. Both at the apical and basal portions the lateral mesocardial layers come to fusion.

A single myocardial tube of the atria begins at the atrioventricular constriction cranially and continues into the sinus venosus caudally, demarcated by the sino-atrial constriction. This muscular tube shows a marked asymmetry on both sides, for the left side, being decidedly expanded in all directions in comparison with the right side, just contrary to the ventricle, in which the right side is apparently more voluminous than the left side and bulges considerably caudolaterally. Moreover, this opposing asymmetry must be attributed partly to the normal oblique direction of the atrioventricular constriction.

In consequence of this asymmetrical relation, the atrial tube forms a typical curvature with the ventricular limb at the atrio-ventricular junction, so that its convexity is directed toward the left side in the horizontal plane and ventralward in the vertical plane.

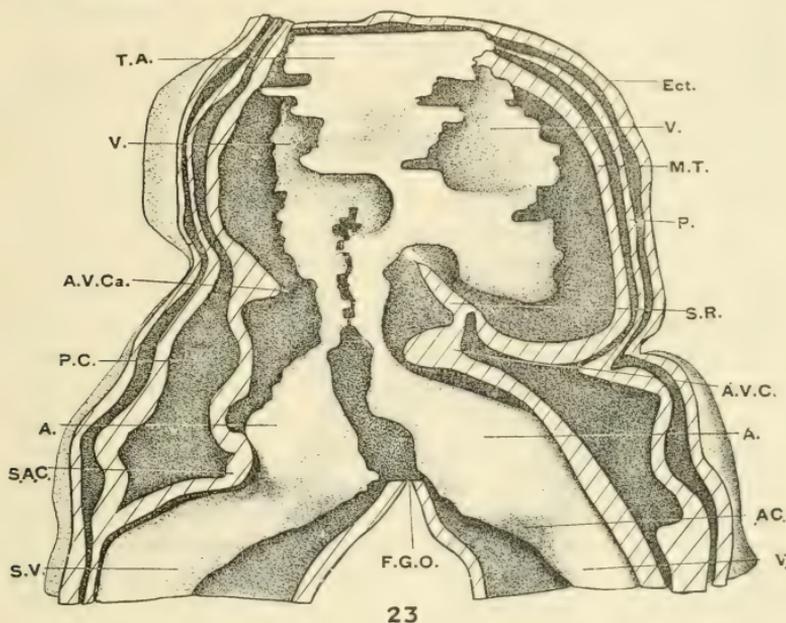


Fig. 23 Dorsal view of the reconstruction of the same embryonic shield (stage VIII) from which figure 22 was drawn. Dorsal wall of the pericardium and of the myocardium have been removed to expose the endothelium, which is subdivided into several individual portions in conformity with the myocardial subdivision. The two lateral endothelial tubes have fused and now communicate with each other throughout a middle third of the ventricle, but elsewhere they are separated. $\times 100$.

The cranial extremity of the atria opens into the left ventricle through the atrioventricular canal, which is situated on the left side from the midsagittal line, for the right atrioventricular constriction is apparently more deeply infolded and proportionately the prominent ridge at the inner wall is strongly pro-

jected into the canal on the right side. The caudal extremity of the atria is continued into the sinus venosus, which diverge from each other into the two lateral myocardial tubes in relation with the foregut opening. The demarcation of these different portions is indicated by the sino-atrial constriction, of which on the left side the myocardial wall is more deeply infolded than on the right side.

The two lateral layers of the dorsal mesocardium are fused together from a middle third of the ventricle to the cranial part of the atria; they are in close contiguity at the atrioventricular constriction, in which region the dorsal mesocardium is beginning to disappear in an embryo slightly older than that of this stage. But at the portion of the bulbus cordis and the caudal part of the atria, the layers of the dorsal mesocardium have not come in contact. The arterial opening is disposed nearly vertically, but slightly to the left side, through which the endothelial tube comes from the myocardial cavity, while the venous opening is disposed dorsocaudally and assumes an irregular triangular space. Its base is situated caudally and ventrally opposite to the foregut opening, as a result of which the layers of the dorsal mesocardium divert together with the corresponding muscular tubes, which continue caudally. Its apex of the venous opening is directed cranialward and lies at the higher horizontal level. Through this intermesocardial space the enclosed endothelial tube can be seen.

On the ventral surface the myocardium is reflected onto the ventral wall of the pericardium at the sinus venosus. Here it can be observed that the mesodermal cells have proliferated to form an appreciable thickening around the endothelial tubes, indicating the future septum transversum.

The lateral endothelial tubes are fused in the middle third of the ventricle to the extent of fifteen sections. In this part they communicate with each other and the endothelial cavity is considerably dilated (fig. 23). This craniomedian part of the endothelial tube is bifurcated into the two cranial horns and two caudal prolongations. The cranial horns extend symmetrically from the cranial wall for a short distance on both sides

and terminate blindly opposite to the cranial myocardial extremity of the ventricle, which is of conical form.

The right caudal prolongation is given off from the right side of its caudal wall and terminates as a short conical projection; its apical terminus is directed opposite to the caudal extremity of the right ventricle, conforming with it. The left caudal prolongation is given off from the left side of its caudal wall and caudally connects with the endothelial tubes of the atrium. These separate from each other and continue farther caudalward. From the origin of this left caudal prolongation caudally to the atrioventricular canal, that is, in the caudal part of the left ventricle, the two endothelial tubes show their smallest size and are very close in contact in some places, while in others they separate into two quite independent tubes with complete walls. Opposite to the atrioventricular canal the two endothelial tubes above mentioned begin definitely to separate into two lateral atrial endothelial tubes, increasing their calibers gradually caudalward, while they lie approximately in a parallel direction.

The caudal extremities of the atrial endothelial tubes continue immediately into the endothelial tubes of the sinus venosus and then into those of the vitelline veins. The transition from the atrial endothelial tubes into those of the sinus venosus is marked by a sudden diminution in their diameter together with the general decrease of their calibers and the abrupt lateral divergence of their course, due to the intervention of the foregut opening. These transitional points correspond to the external groove of the sino-atrial constriction.

In the ventricle the endothelial tubes are separated from the myocardial wall by a wide intervening space. In the atrium the intervening space becomes considerably narrower, and finally in the sinus venosus the endothelial tubes are enclosed intimately by their own independent myocardial wall, so that no appreciable space can be seen.

The endothelial tube of the bulbus cordis extends from the dorsal surface of the right ventricular endothelium as its continuous prolongation. This endothelial tube proceeds at first

dorsocranially and then slightly toward the left side. This is enclosed by the corresponding myocardial wall of the bulbus cordis, which is closed cranially, but caudally opens and communicates with the ventricular cavity, as already mentioned. The right ventricular endothelium, which gives off the endothelial tube of the bulbus cordis, is fused together with the left one, but the left part of the fused ventricular endothelium participates in no way with the bulbus cordis.

The endothelium of the truncus arteriosus continues farther dorsally and slightly toward the left side from the bulbus cordis and passes through the above-mentioned arterial opening, and then bifurcates into lateral symmetrical branches, which are located between the foregut floor and the lateral dorsal mesocardial layers and continue farther cranially into the ventral aortae.

The first aortic arch and the ventral aortae are completely formed and the dorsal aortae are considerably elongated caudward.

SUMMARY AND CONCLUSION

In our observations the first sign of the formation of angioblasts is shown in stage I, embryo A, in which neither the head fold nor the anlage of the pericardial cavity has yet appeared.

On the ventral surface of the mesoderm of the splanchnopleura of the cranial portion, cell bands first begin to separate, which separation is more advanced in the embryos B and C. These cell bands are regarded as angioblasts and they are frequently found to adhere to the indented and loosened mesoderm of the splanchnopleura by broader or narrower protoplasmic bridges. It has frequently been pointed-out that mitotic figures are found in the mesoderm of the splanchnopleura in the neighborhood of angioblasts. Furthermore, in many cases where the angioblasts are in close contact with the mesoderm of the splanchnopleura, it is impossible to discriminate the angioblasts from the mesodermal cells of the splanchnopleura, as concerns their sizes, forms, staining reaction, and the form of the nuclei, while a great difference can readily be recognized between the angio-

blasts and the adjacent entodermal cells. These findings show that in the genetic origin the angioblasts for the future endocardium are derived directly from the mesoderm of the splanchnopleura. The origin of the angioblasts from the mesodermal cells continues until a later stage, in which the greater part of the endothelial tubes are already differentiated from the angioblasts in the anterior portion of the embryo, but the origin of the angioblasts can be recognized in the posterior part of the embryo, as is shown in the embryo of stage III.

In their well-known work on pericardial development, Strahl and Carius found it impossible to decide whether the embryonic coelom in the guinea pig appears at first in the region of the heart anlage, proceeding forward into the pericephalic mesoderm, or whether it begins first in the pericephalic mesoderm and then spreads out caudally. They speak as follows: "Doch können wir augenblicklich eine ganz sichere Entscheidung nicht geben." The cause of this ambiguity is that they began their investigation of the origin of the intraembryonic coelom at too late a stage.

For the dog, Bonnet states that, concerning the origin of the intraembryonic coelomic space, that the lateral pleuropericardial cavities, having already distinctly appeared, anticipate the formation of the pericephalic space. To quote Bonnet directly: "Eine ebensolche Spaltung des Mesoderms führt in VIII $\bar{5}$ gleichzeitig im Bereiche des Herzwulstes zur Bildung der Pleuro-Pericardialhöhle. Ihre zuerst paarig angelegten spalten vergrössern sich, vereinigen sich nach vorne und bilden so ein nachhinten offenes Hufeisen; den pericephalen und lateralen Teil der Pleuro-Pericardialhöhle."

In our specimens, embryo C, stage I, shows the discontinuous formation of the intraembryonic coelomic spaces in the cranial portion of the embryonic shield, as also in the pericephalic mesoderm, these spaces beginning as multiple foci. But they are primarily absent in the middle portion of the pericephalic mesoderm.

In stage II, in which the head fold of the embryo begins to separate from the surrounding blastoderm and the foregut has

just begun to develop, the intraembryonic coelomic space spreads out cranially into the pericephalic mesoderm, cleaving the mesodermal layer in such a way, that the lateral primitive pericardial cavities communicate with each other. In a just slightly younger embryonic shield than this, each lateral pericardial cavity has progressed cranially into the pericephalic mesoderm, showing in this place a slit-like space, which however, is divided by a thin mesodermic bridge in the middle line.

The pericardial cavity, therefore, commences simultaneously in the multiple foci, separating irregularly by mesodermal bridges throughout the lateral plate in the cranial portion of the embryonic shield and in the pericephalic mesoderm. These multiple coelomic spaces become confluent to form a single pericardial cavity, having an inverted-U shape, when the mesodermal bridges at the middle line of the pericephalic mesoderm have ultimately disappeared and, in consequence, at this time the bilateral pericardial cavities, already widely confluent, communicate from side to side (stage II, A). In this embryonic shield relatively wide endothelial tubes are differentiated only in the region of the hindbrain plate, where the pericardial cavity is wide open and the mesoderm of the splanchnopleura is thickened, projecting into the pericardial cavity as a prominent fold. In the pericephalic portion of the mesoderm, however, the pericardial cavity is seen merely as a lineal cleavage. Here a few angioblasts are scattered between the slightly thicker mesoderm of the splanchnopleura and the underlying entoderm.

In stage III the bilateral myocardial folds become quite prominent, so that in the region of the hindbrain plate they have been almost converted into the myocardial tubes, and enclose the well-developed endothelial tubes.

The formation of these myocardial folds has progressed cranialward opposite to the foregut opening on the left side. At the same time the endothelial tube becomes gradually thinner cranialward and terminates slightly caudad to the foregut opening. On the right side the formation of the myocardial folds proceeds still farther cranially into the caudal part of the craniomedian limb of the pericardial cavity, where the thicker mesoderm of

the splanchnopleura is raised from the underlying entoderm and in the space between them a number of angioblasts are distributed. On the right side the endothelial tube terminates cranially just opposite to the foregut opening. In front of the cranial termination of the lateral endothelial tubes a number of angioblasts are scattered, so that the cranial extremities of the endothelial tubes are nearly connected with each other through these angioblasts.

The cranial extremities of the lateral myocardial folds have not yet come to complete confluence, as the mesoderm of the splanchnopleura, slightly cephalad to the cranial extremity of the left myocardial fold, remains still in loose contact with the underlying entoderm. If this portion of the mesoderm of the splanchnopleura were completely raised from the underlying entoderm, forming the myocardial fold, then the myocardial anlagen would come into confluence.

The most prevalent opinions with regard to the mode of the formation of the unilateral myocardial heart anlage from the bilateral myocardial tubes agree that, as above described, the bilateral myocardial tubes, at first independently, come to actual fusion with each other, and then the septal wall between them is absorbed secondarily, thus forming a single myocardial cavity. Our specimens show that the formation of the myocardial folds does not occur synchronously throughout the pericardial cavity, as in the region of the hindbrain plate they first appeared and developed considerably, while in the craniomedian limb of the pericardial cavity the formation of the myocardial folds was just starting and rising slightly from the underlying entoderm. However, the communication of the lateral myocardial tubes is accomplished when the formation of the myocardial folds is completed in the craniomedian limb of the pericardial cavity, in which region the formation of these folds occurs last. For this reason, the cranial prolongation of the lateral myocardial tubes has not been brought about by the direct extension of the first part of the myocardial tubes, but by the continuous progressive differentiation into the craniomedian limb of the pericardial cavity. Therefore, the confluence of

the myocardial tubes into a single myocardial cavity is not accomplished by the actual fusion of the bilateral myocardial tubes, followed by absorption of the septal walls.

In stage IV the formation of the myocardial fold is completely accomplished in the craniomedian limb of the pericardial cavity, elevating the mesoderm of the splanchnopleura from the underlying entoderm and projecting into the pericardial cavity. Both lateral myocardial tubes communicate with each other in this region.

Both the myocardial tubes are quite voluminous. They dilate in all directions, especially in their cranial portions, and they gradually reduce their dimensions caudalward. On the surface of these transitions no distinct demarcation can be noted.

The two lateral endothelial tubes are well developed, lying side by side together in the portion of the confluent myocardial cavity, and here independent, intermediate endothelial cells are scattered between the lateral endothelial tubes.

In stage V the cranial portions of the two lateral myocardial tubes show much more dilatation and elongation, as their extent from the foregut opening to the cranial extremities considerably increases. But their confluent part still remains short. The transition from the cranial dilated myocardial tubes to their caudal slender portions is marked by a distinct annular atrio-ventricular constriction, which appears clearly first in this embryo.

A number of workers declare that the lateral myocardial tubes are subdivided into many individual portions by the demarcations prior to the fusion of the lateral myocardial tubes: in the chick, Duval, '99; in the cat, Martin, '02; in the rabbit, Kölliker, '84. Kölliker states, "Ein Herz aus diesem Stadium ist sehr verschieden von dem primitiven Herzen eines Hühnerembryo, was einfach darin begründet ist, dass, wie bemerkt, bei Säugethieren schon vor der Verschmelzung der beiden Herzhälften die drei Herzabschnitte angelegt sind."

Mollier declares in similar language: "An den beiden Herzröhren ist aber kurz vor ihrer Vereinigung schon eine Gliederung bemerkbar."

In our specimens this embryo shows first the atrioventricular constriction, even though in the previous embryo two myocardial tubes were already confluent into a single myocardial cavity.

The two lateral endothelial tubes are considerably developed, especially in the confluent portion of the myocardial cavity, where they approach each other almost to contact. From the atrioventricular constriction caudalward the two endothelial tubes assume an abruptly narrow caliber, thus distinguishing the larger cranial ventricular from the smaller caudal atrial portion. In tracing farther caudally, no demarcation can be detected on them.

In stage VI the confluent myocardial cavity increases especially in the craniocaudal extent. The cause of this may be attributed partly to the retirement of the wedge-shaped septal ridge cranialward, which had projected into the confluent myocardial cavity, as the conversion of the inner walls of the cranial lateral myocardial extremities, and partly to the active backward progress of the foregut opening. Approximately, in the whole extent of the ventricle and in the cranial part of the atria the two lateral myocardial cavities communicate with each other and these two myocardial portions are demarcated sharply by the atrioventricular constriction. In the middle third of the ventricle the two lateral endothelial tubes are actually fused and communicate with each other for a short distance. At the atrioventricular constriction the calibers of the two endothelial tubes are considerably reduced and, as we proceed still farther caudally, they are again gradually increased in diameter.

In an embryo of *Perameles nasuta* having fifteen to sixteen somites, Miss Parker pointed out that the fusion of the lateral endothelial tubes first took place. The fused portion already extends throughout about eighteen sections. She states that from this portion the *bulbus cordis* is derived.

Wang reports concerning a ferret embryo, having thirteen to fourteen somites, that the two endothelial tubes had united in a part of their extent. The fused portion, extending throughout about sixteen sections, appeared to be the ventricular part.

In our specimens this embryo shows first the fusion of the two lateral endothelial tubes throughout only seven sections, having a 5μ thickness, and this united part corresponds to a middle third of the ventricle and lies precisely on the midsagittal plane. In the guinea pig the fusion of the lateral endothelial tubes takes place at a relatively early stage of development—a stage in which in the myocardial and endothelial tubes there can be distinguished simply the ventricular and atrial portions. In the above-mentioned animals investigated by other authors, the fusion of the lateral endothelial tubes was first noted in the relatively older embryo, in which the different parts of the myocardial and endothelial tubes are already definitely subdivided. Moreover, their embryos show that the fused portion is considerably extended in comparison with this embryonic shield.

The factors which are generally accepted as the cause of the loop formation of the endothelial tubes depend on the fact that the rate of growth of the two endothelial tubes exceeds that of the pleuropericardial cavity. Bonnet depicts a dog embryo in which the primary subdivision of the endothelial tubes into sinus venosus, atrium, and ventricle has occurred before they have fused to form a single myocardial cavity.

Wang pointed out the loop formation with the subdivision of the heart (atrium, ventricle, bulbus, etc.) in the ferret embryo, before the endothelial tubes had become fused.

But in our specimens there is no loop formation, nor can the subdivision of the heart be marked out on either of the endothelial tubes before they are fused together, even though the ventricle and atrium may be roughly distinguished by their difference in size.

Contrary to the above-mentioned assumption that the loop formation of the endothelial tubes has been brought about, the moment of fusion of the two lateral endothelial tubes shows quite other facts in the guinea pig. In the embryonic shield at this stage of development the confluent part of the myocardial tube grows excessively in the cranio-caudal direction and decreases its lateral width in comparison with the embryo of stage V, as measured and compared on both reconstruction

models which had been magnified to the same degree. Consequently, the two endothelial tubes are brought together in the median plane, where they come to fusion, by the extreme longitudinal stretching of that part of the myocardial tube in which the endothelial tubes are enclosed. Concurrently, the active dilatation of the two endothelial tubes plays a part in bringing about the fusion, which takes place first in the most dilated portions.

In stage VII the myocardium presents cranially a single expanded craniomedian extremity, assuming a sac form, and here represents the ventricle, while caudally this myocardial sac is bifurcated into two rather slender myocardial prolongations. Their demarcation is indicated by the well-developed atrioventricular constriction slightly cephalad to the foregut opening. The transition from the atrium into the sinus venosus is marked by an indefinite indentation on the bilateral myocardial tubes caudad to the foregut opening and slightly more distinct on the left side. Corresponding to these myocardial constrictions, there can be pointed out a similar indentation on the endothelial tube on the left side.

The two lateral endothelial tubes are fused together in the cranial two-thirds of the ventricle, and its cranial extremity is terminated as a single conical apex opposite to the cranial end of the myocardial ventricle. In tracing farther caudalward from this united portion, the two endothelial tubes are separated.

In this stage of the development the myocardial and endothelial heart anlagen of the ventricle present a considerable asymmetry, due to the unequal growth of the individual parts, despite the fact that, in the embryonic shields prior to this stage of development, the heart anlagen are shown as a practically symmetrical development on both sides, even after the fusion of the lateral endothelial tubes has already been accomplished.

Miss Parker describes the heart of the *Perameles obesula* stage V as follows: "In the ventricular region of the heart, the right and left endothelial tubes are approximately equal in size, but where there is an inequality the right is the larger."

In the ferret embryo Doctor Wang says: "It has been found that the two tubes, prior to fusion, appear to have been shifted as a whole toward the right side and that they remain in this position even after partial fusion has taken place."

In our specimens the myocardial asymmetry of the ventricle is attributed partly to the extraordinary bulging of its right wall dorsally, laterally, and slightly caudally, and partly to the deeper infolding of the myocardial wall of the atrioventricular constriction of the right side. In the ventricle the endothelial tubes are much more dilated on the right side than on the left throughout its whole length, regardless of the fused or non-fused portions, and it expands considerably dorsally, laterally, and caudally. Consequently, they deviate from the middle plane toward the right side and are situated more to the right side of the myocardial ventricle. The right endothelial tube is elongated dorsally at the caudal third of the ventricle, where the two lateral endothelial tubes, being separated, pass through the intermesocardial space vertically. From this portion the *bulbus cordis* develops in a later stage; its termination is cranially bifurcated into two lateral endothelial branches, which continue farther cranialward into the ventral aortae.

In stage VIII there are present three distinct constrictions on the tubular myocardial surface, infolding the whole thickness of the myocardial wall into the myocardial cavity. Consequently, the myocardium can be subdivided by very distinct demarcations into the *bulbus cordis*, bulboventricular constriction, ventricle, atrioventricular constriction, atrium, sino-atrial constriction, and sinus venosus in the craniocaudal succession.

The *bulbus cordis* is demarcated from the dorsal wall of the expanded right ventricle at its cranial end by the horizontal bulboventricular constriction. The bulboventricular constriction shows considerable asymmetry, making a deeper furrow on the external surface of the myocardium at the left and cranial sides, while at the right side it is present as a shallow depression on the external surface, diminishing imperceptibly caudalward, until it has entirely disappeared at the part of the right ventricle. Thus neither external furrow

nor infolding of the myocardium is shown along the caudal boundary of the bulboventricular junction, and here the wall of the bulbus is directly continuous into that of the right ventricle. Corresponding to the external view, the deep infolding of the myocardial wall as the inner prominent ridge is shown at the left and cranial sides of the bulboventricular canal, while on the right side it can be recognized only cranially and disappears insensibly caudalward. In this fashion the curvature of the myocardium at the bulboventricular junction is effected in such a way that its convexity is turned toward the right side. On the dorsal aspect of the bulbus cordis there is a triangular intermesocardial space disposed vertically and slightly toward the left side, through which the truncus arteriosus passes out from the myocardial cavity up to the floor of the foregut.

The atrioventricular canal is well marked and is disposed approximately in the vertical plane. It is produced by the infolding of the myocardial wall, which is deeper and more caudad on the right side than on the left. Consequently, the opening of this canal is situated on the left side of the middle plane. In this relation the myocardial tube forms a marked curvature at the atrioventricular junction, turning its convexity toward the left side and ventralward, with the result that the ventricular portion lies at the right side and slightly ventrally to the atrial portion. This curvature is remarkably accentuated in the next stage of the development, in which, for a short extent, the dorsal mesocardium disappears at the atrioventricular junction and herewith the ventricle comes to the ventral surface of the atrium, being free from the restriction of the dorsal mesocardium.

The ventricle can be divided incompletely into two limbs by the ventral and dorsal longitudinal sulci. At the caudal part of the ventricle, for a short distance, the two limbs are divided into two completely independent cavities by the septal wall. The caudal extremity of the right ventricle terminates blindly as a conical process and it projects caudolaterally.

In the literature I could not find a description of this. On first observation it seemed to me that the septal wall and its conversion into prominent ridges at the inner surface of the ventral and dorsal myocardial wall were produced by the actual fusion of the two lateral myocardial tubes. Therefore, this may account for the remnant of the primitive myocardial septum. But in the embryos of stages VI and VII there was present no septal wall nor prominent ridge similar to this in their single ventricle, in which they would be more distinctly present if they accounted for the production of the actual fusion of the lateral myocardial tubes and the remnant of the primitive cardiac septum.

Accordingly, it appears to be due to the fact that the caudal surface of the myocardial ventricle on the right side is projected actively backward by the unequally excessive rate of growth in this portion, while in the middle plane a part of the myocardial wall does not proportionately accompany this active backward growth, but remains as the septal wall.

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Posición anormal del duodeno.

En el caso descrito por el autor, el duodeno presentaba próximamente la longitud normal, pero estaba plegado de un modo anormal a la derecha de la raíz del mesenterio, delante del riñón. La porción superior es completamente normal. La porción descendente desciende al principio pero después gira dorsalmente delante del hilus del riñón para unirse con un asa semejante de la porción que normalmente forma el segmento transverso. Este último está situado más caudalmente, enfrente del polo inferior del riñón, y su porción terminal corta pasa transversalmente detrás de las raíces de los grandes vasos mesentéricos, a la izquierda de los cuales se une con el yeyuno. Esta unión está situada a la derecha de la columna vertebral, pues la raíz del mesenterio está en el territorio del lado derecho. Las asas anormales del duodeno están insertas delante del riñón mediante el peritoneo. La cabeza del páncreas se extiende inferiormente en el lado mesial a las asas duodenales. El extremo izquierdo del colon transverso forma una larga asa dirigida hacia abajo. El colon ascendente es más largo que lo normal, y el ciego y el apéndice constituyen el contenido de una hernia inguinal oblicua. El autor interpreta el presente caso como un caso de rotación interrumpida o formación incompleta de la curvatura duodenal en el embrión. Veinte casos diferentes de malposición del duodeno han sido citados en la literatura y todos ellos ilustran de uno u otro modo el mismo principio.

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ABNORMAL POSITION OF THE DUODENUM

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SEVEN FIGURES (TWO PLATES)

This case is reported because it shows a rare malposition of the duodenum and is an example of incomplete rotation of this organ around the mesenteric pedicle. There are not many cases of malposition of the duodenum on record. The surgical importance of these anomalies is indicated in the case reported by Mumford ('06) and that reported by Freeman ('20). These cases are of interest because they can be explained on the basis of arrest or variation in the normal developmental processes in that region.

The position of the duodenum in the adult has been studied by many anatomists and surgeons, among whom may be mentioned Braune, Treves, Jonnesco, Schiefferdecker, Dwight, Cunningham, and Ochsner. Dwight ('96-'97) made an extensive study of its shape. Addison ('01) has made an extensive study of the variations of its position.

Meckel, in 1917, and His, in 1885, established the development of the intestinal canal. Mall ('97) has demonstrated how the coils of the intestine develop and the position which they ultimately assume. A comprehensive study of the subject is given by Lewis ('12). Lewis and Papez, in a series of reconstructions designed to show the development of the mesentery, demonstrated how the duodenum rotates around the mesenteric pedicle in young human embryos. More recently, Frazier ('19) has figured a series of reconstructions to show the formation of the duodenal curve, and Vogt ('20) has figured the duodenum and structures in the mesenteric pedicle in human embryos 12.5, 22, and 33 mm. long.

Several embryonic structures shape the duodenal curve. In the young human embryo the mesenteric pedicle is formed dorsal to the gastroduodenal region by the primitive portal anastomosis, the pancreas, and the roots of the mesenteric vessels. At an early stage (5 mm.) the primitive portal anastomosis, the proximal ends of the vitelline vessels, and the hepatic bud form the conspicuous features in this region. Soon, however, the growing pancreas invades this area of the mesentery and forms a constant and prominent mass around the roots of the great mesenteric vessels. It is over the right side of this pedicle that the curve of the duodenum is begun, and is subsequently completed by passing around its dorsal side. Complete rotation of the duodenum is normally concomitant with the rotation of the general mesentery, as shown in a 42-mm. embryo by Papez and Lewis ('17) and in a 40-mm. embryo by Frazier ('19).

REPORT OF CASE

The case (fig. 1) described in this report probably illustrates an arrested or incomplete rotation of the duodenum as observed in an adult male subject (no. 607) in the anatomical laboratory of Cornell University at Ithaca, New York. The viscera of this subject had a healthy appearance and there was a complete absence of inflammatory adhesions. The position, relations, and peritoneal attachments of the duodenum must be regarded as having an embryonic origin.

The stomach was somewhat smaller than normal, but its position, relations, and peritoneal attachments were quite normal. The pylorus had the usual situation just to the right of the midline. There was no shortening of the lesser omentum. No adhesions of the pylorus or duodenum to the liver were present. The case reported by Brash and Stewart ('20) is cited here to show that a reversal of rotation of the stomach and mesogastrium (to the right) produces a marked malposition and flexion of the duodenum, but is not necessarily associated with complete reversion or torsion of the mesentery and intestines. They cite similar cases of transposition of the stomach reported by Lunay

and by Debovie in which the three parts of the duodenum were reported as normal. In the case reported by Bryce ('99) there was a marked constriction of the stomach which he thought might have a causal relation to the duodenal anomaly.

The liver was quite normal excepting that the tip of the left lobe (*e*) was elongated and constricted from the main portion, comparable to the liver described by Harman ('99) in which there was no mention of variations of the duodenum. Since this anomaly of the liver is one often observed, it would not seem to have any causal relation to the malposition of the duodenum. The gall bladder, bile ducts, and other structures in the lesser omentum had the usual disposition.

The duodenum was wholly to the right of the midline coiled in an unusual manner in front of the hilus and lower pole of the right kidney. Its length was about normal. Its superior portion (*sd*) formed a coil convex upward, and had normal relations. Its superior surface was in contact with the quadrate lobe of the liver; it was covered by peritoneum which passed to the left into the epiploic foramen and peritoneum of the lesser omentum and passed downward into the superior layer of the mesocolon. Its left surface lay against the upper portion of the head of the pancreas and against the hepatic artery. Thus, the hepatic fold (inferior pancreaticoduodenal fold) passed along the upper left border of this portion of the duodenum. The hepatoduodenal ligament was attached to the duodenum just to the right of this fold. The gastroduodenal artery passed downward behind the duodenum.

The descending portion of the duodenum formed a coil (*dd*) which turned upward to join a coil of the inferior portion in front of the hilus of the right kidney. The upper and right surfaces of the descending coil were in relation with the inferior surface of the gall bladder and right lobe of the liver. They were covered by peritoneum which passed dorsally to the surface of the right kidney and ventrally into the upper layer of the transverse mesocolon. The left surface lay against the head of the pancreas, common bile duct, and upper extent of the superior mesenteric vessels which separated it from the inferior vena

cava. In front and along the lower border of these duodenal coils passed the right extremity of the transverse colon bound to them by the mesocolon. These coils of the duodenum rested in part upon the right end of the transverse colon. The accessory pancreatic duct could not be located. The duodenal papilla with the openings of the common bile duct and the main pancreatic duct was situated in the dorsomedial wall of the descending coil (*dd*). No duodenal diverticula were found.

The head of the pancreas (*pa*) was molded against the left side of the descending coil (*dd*) and extended for some distance in a caudal direction to the right of the great mesenteric vessels. The pancreaticoduodenal vessels had the usual distribution between the head of the pancreas (*pa*) and descending coil (*dd*).

The third portion of the duodenum (*td*) formed a large upward coil in front of the hilus and lower pole of the right kidney. The ascending end of the coil passed dorsal to the root of the great mesenteric vessels and head of the pancreas to gain the left side of the root of the mesentery where it turned down to join the jejunum (*jj*). This junction (*jj*) was in front of the right psoas muscle to the right of the inferior vena cava opposite the third lumbar vertebra. The coils were covered on the right side by peritoneum which bound them to the surface of the right kidney and to the right parietal wall. The terminal portion as it passed through the root of the mesentery received from it the usual covering. Ventral to the terminal portion was the root of the mesentery (*mes*) with the superior mesenteric vessels and head of the pancreas (*pa*). Ventral to these was the right end of the transverse mesocolon.

The abnormal coiling of the duodenum to the right of the root of the great mesenteric vessels and the right-sided position of the mesentery and duodenojejunal junction were the striking features of this anomaly. They are to be interpreted as being due to an incomplete rotation of the duodenum around the mesenteric pedicle in the embryo.

The root of the mesentery of the small intestine and its usual contents extended along the right surface of the right psoas into the iliac fossa. Its upper extent was about 7 cm. to the

right of the midline. The lower end was elongated and permitted a considerable downward displacement of the ileocaecal junction and caecum which with the appendix formed a large oblique inguinal hernia.

The colon was longer than usual. The left portion of the transverse colon formed a large downward loop in the great omentum. The ascending colon was so elongated as to permit the caecum and appendix to enter the large hernia. In the duodenal anomaly figured by Harman ('98) there was a large downward loop of the ascending colon at the hepatic flexure. It might appear that an overgrowth of the colon with an exaggeration of the torsion of the entire tract may have a causal relation to such duodenal anomalies. However, in Sencert's ('05) case there was an incomplete growth as well as incomplete rotation of the intestinal tract, the caecum remaining in the left hypochondrium. In the case reported by Armstrong ('10) and in Boyd's ('14) case similar conditions obtained.

ABSTRACTS OF REPORTED CASES

In 1886 Schiefferdecker reported two cases in which there was an unusual coiling of the duodenum and first portion of the jejunum in the root of the mesentery.

In 1896 Dwight referred to some curious cases he had seen in which the distal part of the duodenum formed a coil in front of the left kidney, so that the duodenum had the shape of an S placed on its side. His cases were explained as due to an overlong duodenum (Piersol's Anatomy).

Roud, in 1898, reported a case (fig. 6) in an adult in which the first portion of the duodenum was normal, but the remainder suspended in a short mesoduodenum extended downward to the right iliac fossa and did not cross dorsal to the mesenteric vessels. The caecum was in the pelvis. Ascending over the right pelvic brim the colon passed to the left through the root of the mesentery. He recognized that the condition was an anomaly of embryonic origin in which the normal torsion of the intestines had been partially reversed so that the colon passed behind the mesentery of the small intestine.

Harman, in 1898, reported a case (fig. 4) in which there was a coiling of the descending portion of the duodenum and the duodenojejunal junction was to the right of the second lumbar vertebra and turned to the right. This is comparable to case 5 reported by Addison. There was also a large downward loop of the colon at the hepatic flexure, as if it were double. Harman's ('98) case is comparable to the one reported in this paper.

Bryce, in 1899, reported a case (fig. 2) in which the first portion of the duodenum was normal, but the second and third portions formed a long downward U-shaped loop attached in front of the right kidney and to the right of the great mesenteric vessels. The short terminal portion passed dorsal to the mesenteric vessels and joined the jejunum opposite the second lumbar vertebra. There was a marked constriction of the stomach. Bryce ('99) thought that the duodenal anomaly might have been produced by a failure of the normal twisting of the duodenum (Young, '98).

Addison, in 1901, reported a case (no. 40) in which the ascending portion was dorsal and somewhat to the right of the descending portion, comparable to Bryce's case (fig. 2). In another case (his no. 7) Addison found the duodenum and the duodenojejunal junction to the right of the midline.

Fawcett and Blachford, in 1904, made 337 postmortem examinations to determine the position of the third part of the duodenum. In four cases the duodenum did not cross the vertebral column, but ran into the rest of the intestine along the right side of the column. From their observations it would seem that this anomaly occurs in more than 1 per cent of individuals.

Sencert, in 1905, reported a case (compare with fig. 5) of incomplete torsion of intestinal loop in a newborn child. The duodenum and head of the pancreas were contained in a short mesentery and were movable. The duodenum was on the right side, formed a simple curve; and its third and fourth portions were absent. The colon was not fully developed; the caecum was in the left hypochondrium. The root of the mesentery was adherent along the midline, as in Eddy's case (fig. 3).

The case (fig. 7) reported by Clermont ('05) was singular in that the first and second portions of the duodenum were to the left of the superior mesenteric vessels, while the transverse and ascending portions were on the right. As in other cases, the terminal portion was short and passed dorsal to the great mesenteric vessels to join the jejunum. In this case it appears that there was a partial reversal to the left of the normal duodenal loop.

Mumford, in 1906, did a gastrojejunostomy on a case (compare with fig. 4) in which there was an incomplete formation of the duodenal curve. The duodenojejunal junction was so far to the right that, as the stomach filled, it pulled away from the jejunum at the site of the operative union. He emphasizes the importance of recognizing such anomalies.

Reid, in 1908, reported an incomplete torsion (fig. 5) of the intestinal loop. In his case the whole colon, quite fully formed in length, remained on the left side. The duodenum was unusually mobile. It was coiled on the right side of the great mesenteric vessels and there joined the jejunum.

Armstrong, in 1910, reported a case (compare with figs. 5 and 6) in which the duodenum extended to the right of the hepatic flexure and ascending colon, and joined the jejunum below the caecum. With the head of the pancreas it was suspended in a fairly long mesentery. "The condition was clearly one of arrested development."

Eddy, in 1912, reported a case (fig. 3) in which the duodenum was comparable to the case reported by Reid. Although the caecum was in the right iliac fossa, the larger part of the colon was on the left side. In this case the rotation of the primitive loop of gut was arrested, so that the intestine persisted in its primitive relation. The pancreas consisted of two parts.

Boyd ('14) reports a case (compare with figs. 5 and 6) in which the third and fourth portions of the duodenum were absent. The duodenum joined the jejunum under the hepatic flexure of the colon. The jejunum commenced as coils on the right side and extended into the right iliac fossa.

Freeman ('20) reported a case (compare with fig. 2) of a long U-shaped duodenum with a kink and a constriction at the duodenojejunal junction.

Brash and Stewart ('20) reported a case of right-sided rotation of the stomach, spleen, and mesogastrium in which the normal duodenal curve was markedly altered (compare with fig. 7).

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ABBREVIATIONS

<i>a c</i> , ascending colon	<i>mes</i> , mesentery
<i>a d</i> , ascending duodenum	<i>m d</i> , mesoduodenum
<i>b d</i> , bile duct	<i>o b</i> , omental bursa
<i>c l</i> , colic ligaments	<i>pa</i> , pancreas
<i>d c</i> , descending colon	<i>py</i> , pylorus
<i>d d</i> , descending duodenum	<i>s d</i> , superior duodenum
<i>e</i> , tip of left lobe	<i>s m v</i> , superior mesenteric vessels
<i>g b</i> , gall bladder	<i>st</i> , stomach
<i>i v c</i> , inferior vena cava	<i>t c</i> , transverse colon
<i>j j</i> , jejunal junction	<i>t d</i> , transverse duodenum

PLATE 1

EXPLANATION OF FIGURE

1 Abnormal position of the duodenum in an adult man. The coiling of the descending and transverse portions in front of the kidney are the chief features. The root of the mesentery was on the right side.

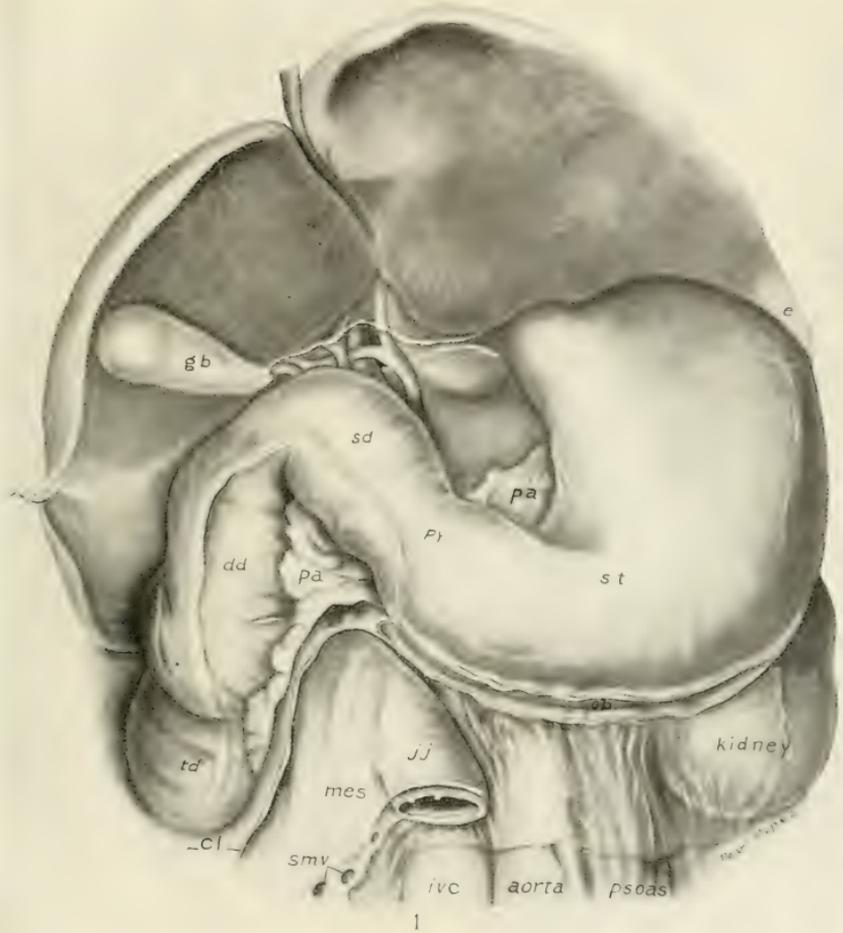
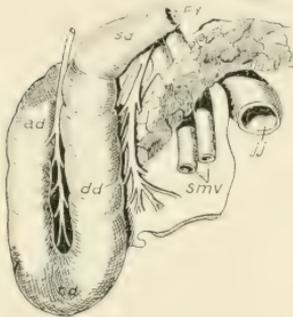


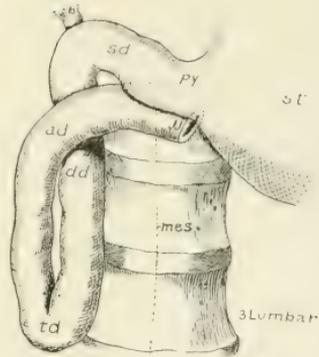
PLATE 2

EXPLANATION OF FIGURES

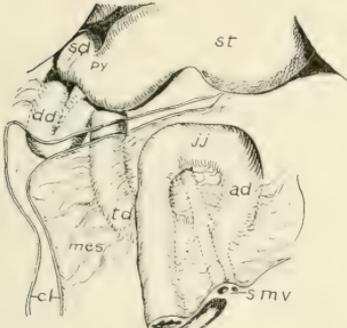
- 2 Copy of Bryce's case. Freeman's case was similar, but the ascending limb of the U was to the left as in U forms described by Dwight.
- 3 Copy of Eddy's case. Compare with figures 5 and 6.
- 4 Copy of Harman's case. Mumford's case was similar.
- 5 Copy of Reid's case. Boyd's case was similar. Sencert's case in a newly born child was similar, but caecum was in left hypochondrium.
- 6 Copy of Roud's case. Armstrong's case was similar. Both resemble cases included in figure 5.
- 7 Copy of Clermont's singular case. The case of Brash and Stewart was similar, but stomach, spleen, and mesogastrium were rotated to the right.



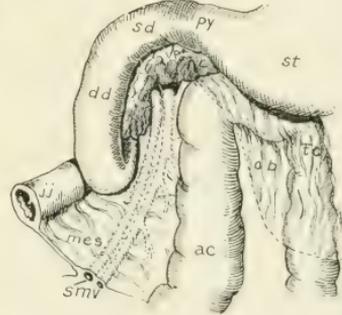
2 Bryce



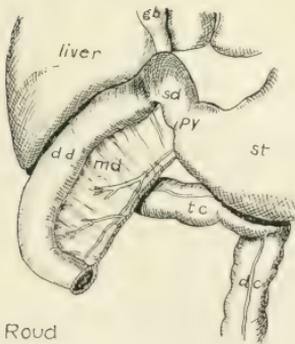
3 Eddy



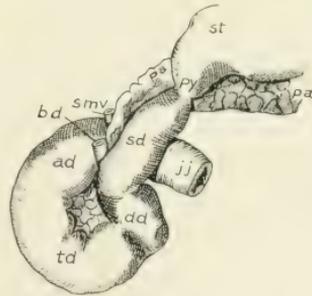
4 Harman



5 Reid



6 Roud



7 Clermont

Resumen por el autor, Wesley M. Baldwin,
Union University Medical College, Albany.

Un estudio sobre la profundidad de penetración de la energía los rayos de luz ultravioleta en el embrión del renacuajo.

En la presente investigación el autor ha descubierto que la luz de rayos ultravioleta de una longitud de onda comprendida entre 214.4 y 240.5 $\mu\mu$ tiene tan solo un poder de penetración de 0.02 mm. en el embrión del renacuajo. Toda la energía, en otras palabras, es absorbida por las dos capas de células ectodérmicas de los embriones durante el periodo de oclusión del tubo neural. La energía luminosa produce una reacción intracelular caracterizada por una ausencia de fenómenos vasculares de inflamación, por esferulación de las células y retardamiento del proceso de reparación, sin ir acompañados de extrusión o exfoliación.

Translation by José F. Nonidez
Cornell Medical College, New York

A STUDY ON THE DEPTH OF PENETRATION OF ULTRAVIOLET LIGHT RAY ENERGY IN THE EMBRYO OF THE TADPOLE

W. M. BALDWIN

Union University (Albany) Medical College

The author has previously detailed the effects of ultraviolet light ray energy acting upon the frogs' fertilized ovum during the early stages of development.¹ It was the purpose of the experiment to ascertain the localization of the organ-forming substances in the egg and incidentally the penetrative capacity of the rays. It is no new fact to point out in connection with the latter that radiant energy of this wave length is comparatively slightly penetrative. Henri² has measured the absorption capacity for wave lengths varying from 214.4 to 240.5 μ , and ascertained that protoplasm absorbed nine-tenths of the energy with thickness of 3.8 μ and 79.0 μ , respectively. The apparatus used for the experiments and described in the previous papers furnished a wide range of these wave lengths. The observed results seemed to indicate that only a relatively small mass of protoplasm superficially placed in the egg was so chemically altered by the energy as to be unable to participate in the normal developmental changes of the embryo. While it was difficult to ascertain the exact depth of penetration of the rays, the fact seemed to be demonstrable that the lethal action was restricted to a depth certainly no greater than 0.1 mm.

A more extended study of the action of the rays was carried out subsequently upon embryos of the tadpole in more advanced stages of development for the express purpose of determining

¹ Baldwin, *Anatomical Record*, vol. 9, no. 5, May, 1915, and *Biological Bulletin*, vol. 37, no. 5, November, 1919.

² v. Henri, Des Bancels, and Wuermsler, 1912, "Etude de photochimie biologiques," Paris: Masson.

in terms of cell layers the depth of active penetration of the rays. A further desideratum consisted of the possibility of elimination of a certain error of absorption through the complete removal of the egg envelopes and of their investing jelly which had proved to be fluorescent and highly absorptive of light energy. The embryos which were used were selected at the time of closure of the neural tube. Several series of embryos served as controls for each experiment. About 100 specimens were rayed, of which thirty were sectioned serially and carefully studied. The localization of the activity of the rays was effected through the interposition of a tinfoil diaphragm between the specimen and the arc emitting the rays. A pinhole in the diaphragm permitted a beam of light 0.3 mm. in diameter to impinge upon the surface of the embryos. The intensity of the light was increased through the employment of two powerful quartz convex lenses placed close to the arc. The exposure time varied with the experiments. In all instances, however, it was continued until a whitish spot made its appearance upon the embryo; in other words, until the pigmented ectoderm became distinctly blanched.

An exposure prolonged beyond this characteristic reaction resulted in the production of a marked and sharply delimited swelling rapidly assuming globular proportions which in the course of a few hours was followed by an exovation. The specimens were afterward fixed according to Schultze's formalin method at periods ranging from fifteen minutes to thirty-four days after the exposure.

Microscopic examination of the sections demonstrated beyond question that the ultraviolet light rays had produced an injury which was wholly confined to the two-layered ectoderm of the embryo. The underlying mesodermal cells were normal in every respect. Upon careful measurement, the normal ectoderm was found to have a thickness averaging about 0.02 mm. over the region rayed. The absorption capacity of these superficial cells for the energy was sufficient to effectively protect the underlying mesodermal cells from the injurious action of those rays which filtered through them.

It would seem from the data given that, while diverse results are obtained through the use of this energy, these are referable chiefly to effects produced in and restricted to the superficial layers of the embryo. This is admittedly no new contribution to our knowledge on the subject, yet here is obviously demonstrable evidence of the complete absorption of practically all of the injury-producing energy by a thin two-cell layer of ectoderm with the concomitant protection afforded to the underlying cells by this procedure. The dosage gives an indication, moreover, of what might be termed the absorption capacity of the ectodermal cells compatible with their physiologic activity. A slight increase either in the time of exposure or in the concentration of the rays, the raying distance remaining constant, would have induced the death of the constituent cells. This was repeatedly verified during the experiments.

Again, it is difficult to deny that some of the more penetrative or 'harder' rays of physiologic activity must have reached the underlying mesodermal cells, yet the absence of morphologic modification and the want of evidence of developmental interruption among the cells of the mesoderm and of its derivatives both seem to argue the presence of so small an amount of this energy as not sufficiently potent to leave even a temporary indication of its reaction effect. In this connection it may be well to point out the capacity of the organism to rectify by means of a delayed process of chemical postgeneration whatever change may have been brought about by this form of energy such as was referred to in the before-mentioned earlier papers.

Several interesting phenomena of reaction are evidenced by these superficial cells. A temporary retraction of the chromatophores of the ectoderm, which accounts for the blanching results noted at the time of the experiment, is first manifested. This is followed within an hour by a marked distention of all of the cellular constituents of the ectoderm. This reaction is wholly confined to the cells of the ectoderm and is unaccompanied by the usual picture of blood-cell multiplication or vascular reaction. At the early stages selected for the experiment,

such a phenomenon is naturally accounted for through the absence during such periods of formed blood-elements and of peripheral vessels. No evidence could be obtained at any period following the raying that the maximum reaction was confined to the superficial layer of the ectoderm. So far as the histologic features might serve as a criterion, this was in both layers the same in kind and in degree.

Within this area the ectoderm was increased in thickness to three times that of the controls. A low swelling of uniform height was evident, consequently, in the gross. Histologic sections revealed an extension of this swelling as a downgrowth into the underlying mesodermal tissue. The increase in thickness depended upon two factors; first, an increase in size of the individual cytologic elements, and, secondly, an increase in number of these elements. The cells presented enlarged nuclei and cell bodies. Since the shape of the latter tended toward the spherular, there was a consequent loss of intimate contact between contiguous cell borders. The appearance resembled the phenomenon of cell retraction, and in exaggerated instances led to the formation of continuous, tunnel-like spaces which extended relatively great distances between the ectodermal cells. Such have been noted before by investigators studying the biological effects of radiant energy and, by the author as well, with x-rays. The assumption of the spherical outline suggests parenthetically that this feature might well be accounted for on the basis of a disturbance in surface tension of the cells induced by the light energy.

The quantity of pigment, normally restricted for the most part to the superficial surface of the superficial layer of cells of the ectoderm, was found to be greatly increased not only at this usual level, but throughout the entire thickness of the superficial cells and greatly increased in amount relatively in the cells constituting the deeper layer of the ectoderm (sunburn?). The cells of the injured area abandoned their orderly stratified appearance and assumed a heaped-up disorderly arrangement. In the thicker portions of the area as many as five or six irregular layers of these cells could be made out. Such appearances

obtained within twenty-four hours and were observed as late as thirty-days following the raying. Mitoses appeared frequently in the affected area after the first ten-day period, but at no time could amitotic division of cells be identified, thus confirming Poynter's observations on wound repair in embryos.³

Resolution was unaccompanied by vascular reaction at all periods of the process. The cells resumed their normal morphologic features gradually. The normal, orderly, stratified arrangement in cell layers was very gradually resumed by a process not at all clearly understood at the present time. Neither exfoliation of cells nor the features of cytolysis were at any time evident. Protoplasmic or nuclear extrusions were not detected. Whatever change had been artificially wrought appeared to be of the nature of an intracellular reaction, and as such would seem to be comparable to that brought about by x-rays. A lethal amount of energy had not been absorbed apparently by any cytologic constituent to so great a degree as to render it unfit for the subsequent chemical ontogeny of the cell. The damage was repaired by the cells as individuals and not by the cell mass as a whole. There was no interposition of a repair process on the part of the organism. The protraction of the restorative process is, furthermore, strongly reminiscent of the effects both of x-ray and of radium energy.

³ Poynter, *Anatomical Record*, vol. 16, no. 1, March 20, 1919.

Resumen por el autor, Emanuel Blankfein,
New York University.

Un ejemplo de disociación de las ramas de la arteria femoral profunda.

En el caso descrito por el autor la arteria circunfleja media se origina en una rama de la femoral que forma un tallo de origen común para ella y para las arterias perforantes primera y segunda. Las arterias perforantes tercera y cuarta también nacen de un tallo común, mientras que la circunfleja lateral nace independientemente de la femoral. Esta disposición de vasos que ordinariamente proceden de la arteria profunda no parece haber sido descrita previamente, aun cuando se han publicado tres casos algo semejantes.

Translation by José F. Nonidez
Cornell Medical College, New York

AN EXAMPLE OF DISSOCIATION OF THE BRANCHES OF THE A. PROFUNDA FEMORIS

E. BLANKFEIN

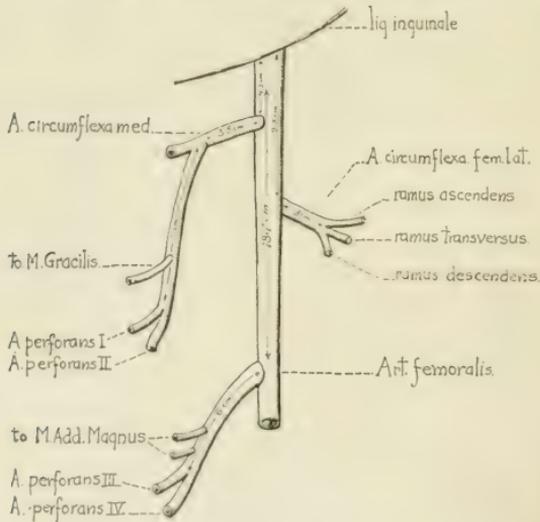
*Anatomical Laboratory, University and Bellevue Hospital Medical College,
New York University*

Although the circumflex arteries often fail to take origin from the profunda in accordance with the traditionally accepted description, the perforating arteries very seldom do so. Three cases are recorded in the literature in which the aa. perforantes have not arisen from a profunda artery of the usual type, and a fourth example of the anomaly has come recently under observation. The arrangement of the perforating arteries of the case in question differs from that of the cases already published, which differ also from one another. This fourth case, therefore, would seem to be worthy of publication.

The recorded cases are those published by Young (1), Ruge (2), and Hepburn (3) in 1879, 1895, and 1896, respectively. The medial and part of the lateral circumflex arteries of Young's case are described as having arisen from the femoral by means of a common stem of origin; the decending ramus of the lateral circumflex and the only two perforating arteries present arising separately. In Ruge's case the first perforating artery arose from the medial circumflex, the second from the lateral circumflex, while the third arose from the femoral. The two circumflex arteries of Hepburn's case arose from the femoral by means of a common stem of origin. The origins of the first and second perforating arteries were combined in a similar manner, as were also those of the third and fourth perforating arteries.

The arteries of the right thigh of the case that forms the subject of the present communication were distributed in the usual way. The left femoral artery gave origin to three large branches in addition to the a. genu suprema, and the usual small inguinal and muscular branches. The first large branch arose 4.5 cm. below the inguinal ligament and after a course

of 3.5 cm. divided into a medial circumflex artery of the ordinary character, and a branch that gave origin to the first two aa. perforantes. The latter branch passed posteriorly to the adductor longus muscle, and was related to its accompanying vein in the same manner as the normal profunda artery; it gave a small branch to the gracilis. At 9 cm. from its place of origin, it divided into the first and second perforating arteries, both of which pierced the adductor brevis and magnus muscles. The second branch, about the same size as the first, was the lateral circumflex artery, it arose 9.5 cm. from the inguinal ligament. The third branch, about double as large as either of the others, arose 18.7 cm. beyond the inguinal ligament, and was unaccompanied by a vein. After having given origin to two small branches to the adductor magnus, it divided 6 cm. from its origin, into the third and fourth perforating arteries, which pierced the adductor magnus to be distributed to the hamstring muscles.



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- 1 YOUNG, A. H. 1879 Abnormal arrangement of the branches of the femoral artery. Jour. Anat. and Phys., vol. 13.
- 2 RUGE, G. 1895 Varietäten im Gebiete des Arteria femoralis des Menschen. Morph. Jahrb., Bd. 22.
- 3 HEPBURN, D. 1896 Double profunda femoris artery. Jour. Anat. and Phys., vol. 30.

Resumen por el autor, Zeno Payne Metcalf,
North Carolina State College and Experiment Station.

Algunas notas de laboratorio.

En el presente trabajo el autor discute brevemente algunos métodos técnicos de laboratorio. Recomienda el papel bromuro en vez de placas de vidrio para obtener negativas para reconstrucciones en placas de cera, evitándose de este modo el hacer positivas, puesto que las negativas en el papel bromuro sirven para todo propósito. Para placas de cera el autor recomienda el uso de las hojas de cera pura de abejas obtenidas para la manufactura de los panales, principalmente porque es fácil de procurar y es barata. El autor describe y representa un microscopio simplificado para el dibujo, provisto de una lámpara Mazda. Este microscopio presenta sobre otros microscopios similares la ventaja de que con él se obtiene una serie extensa de aumentos con un pequeño número de lentes. También describe el autor una escala duplicadora, que se representa en el trabajo. Esta escala se emplea para para representar el lado complementario de un dibujo bilateral.

Translation by José F. Nonidez
Cornell Medical College, New York

SOME LABORATORY NOTES

Z. P. METCALF

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THREE FIGURES

The following notes on laboratory practice are offered for the benefit of other workers. No claims of originality are made, but the writer has frequently noticed that little individual laboratory practices are of use to other workers and frequently have a wider application than is usually appreciated. Neither is it intended to imply that the methods here discussed are the best methods, but if these notes stimulate any one to produce better methods they will have served their purpose.

SOME POINTS ON WAX-PLATE RECONSTRUCTION

Lewis (*Anat. Rec.*, vol. 9, pp. 719-729, 1915) has recently given some very valuable points on reconstruction, but the following points seem worthy of mention. Instead of using glass plates for negatives, it is much easier, more direct, and cheaper to use bromide-paper. The bromide-paper negatives can be used instead of positive prints from the glass negatives, as it really makes no difference in reconstruction whether the photograph we are using is a negative or a positive. Hence all that is necessary is to make the bromide-paper negative and then transfer it to the wax plate. Bromide-paper is handled very much like a glass plate, or the popular gas light papers, such as Velox, Cyko, Artura, etc., but as detailed directions are enclosed with each batch of paper it is not necessary to give them here. Thus we simplify the process greatly and in addition cheapen the process very much, for the large-size plates used are rather expensive. The paper negatives are convenient to store and are not easily injured. Bromide-paper is to be preferred, too,

because it gives flatter, softer negatives than most plates, and because it can be handled in a strong yellow light with ease and comfort. Most types of plate-holders used with photomicrographic cameras are fitted to take either plate or bromide-paper, and if one has such an apparatus no modifications need to be made. If one is provided with a projection microscope only and desires to use the darkened laboratory as a camera, it is very easy to arrange a printing frame with a clear glass plate so that the image can be projected onto it. The printing frame is then used to carry the bromide-paper. A possible objection to the bromide paper is that it requires a somewhat longer exposure than an ordinary plate, but when it is remembered that it is not necessary to make a positive from this negative it will be seen that the process is really very much shortened.

The making of wax plates is a rather tedious, laborious process, but if the plates are purchased from dealers they become very expensive especially if they are to be used by beginners. Hence I was very much interested to know that in the process of manufacturing comb honey and other foundation the beeswax was first smoothed out in a fairly uniform sheet and to know that these sheets could be purchased from foundation manufacturers at a cost very little in advance of the cost of raw beeswax. While these sheets are not absolutely uniform in thickness and while they are not a stated number of millimeters thick, the fact that they can be bought very cheaply compared to the reconstruction wax sheets leads me to believe that they are deserving of recognition, especially by the beginner. Perhaps too much emphasis has been laid upon the fact that wax sheets must be absolutely uniform in thickness. It is well to remember that reconstruction is really drawing in three dimensions and that there may be other errors besides the errors in the thicknesses of the wax plates used. From two manufacturers I have been able to secure three thicknesses of wax called by the manufacturer sheeter wax, brooder sheets, and plain sheets. These were cut into pieces 12 x 12 inches. The sheeter wax averages 2.15 mm. in thickness, the brooder sheets average 1.23 mm. in thickness, the plain sheets average 4 mm. in thickness. It

takes but a very simple calculation to show that if we are reconstructing sections $10\ \mu$ thick and are using a wax plate 2.15 mm. in thickness our magnification must be 100 times 2.15, or $215\times$, if one plate is to be used for each section. If every other section is used, our magnification would be 50 times 2.15, or $107.5\times$, and so for any other combination of thickness of section and thickness of wax plate. The formula is

$$\frac{1000}{\text{thickness of section in } \mu} \times \text{thickness of wax plates} = \text{magnification}$$

required. It is a very small matter to adjust the photomicrographic apparatus for any magnification with the aid of a stage micrometer and a millimeter scale. Thus, if we wish to secure a magnification of 215, a stage micrometer ruled to 0.1 mm. is inserted into the photomicrographic microscope and the lens selected that will give approximately our required magnification; then by moving the ground-glass screen backward and forward we can get a position where the lines of the stage micrometer as projected on the screen are separated by a distance of 21.5 mm., and we have the proper magnification. These odd magnifications are usually as easy to secure as the $50\times$, $100\times$, and $200\times$ that are generally recommended in wax-plate reconstruction.

A DRAWING MICROSCOPE

The drawing projection microscope here described is very simple and easily constructed, and yet it has proved entirely satisfactory and compares very favorably with the much more expensive Edinger drawing apparatus. It has the advantage over the old-style Edinger apparatus of using a mazda lamp instead of an arc. The condenser is the ordinary microscope condenser and the body tube is narrow, hence in these respects it is not as convenient as the Edinger apparatus, but in our work we have found the occasions when we need the double condenser rather rare, and with low-power objectives the condenser may be removed entirely, and by shifting the lamp house fairly satisfactory results can be secured.

This apparatus has combined the following advantages: first, it is vertical; secondly, the lamp and microscope can be readily

adjusted independently of each other; thirdly, it uses a mazda lamp as a light source, thus avoiding the trouble of adjusting carbons; fourthly, the microscope can be brought within 40 cm. of the drawing-board or elevated to a distance of 70 cm. thus giving a great range of magnifications with a limited number of objectives and oculars, as is shown by the following table:

Table of magnification

	DISTANCE MICROSCOPE STAGE TO DRAWING BOARD			
	40 cm.	50 cm.	60 cm.	70 cm.
16 mm. objective front lens removed no eyepiece	9×	12×	15×	18×
16 mm. objective front lens removed 4× eyepiece	13×	21×	29×	36×
16 mm. objective front lens removed 8× eyepiece	23×	36×	50×	63×
16 mm. objective no eyepiece	21×	26×	31×	36×
16 mm. objective 4× eyepiece	38×	58×	78×	98×
16 mm. objective 8× eyepiece	70×	102×	137×	173×
4 mm. objective 4× eyepiece	200×	300×	400×	500×
4 mm. objective 8× eyepiece	350×	510×	680×	850×

In its essential features the apparatus consists of a strong table with an ordinary white-pine drawing-board, for a top. To this table are bolted two square uprights (fig. 1 *u*) 22 x 30 inches, bolted together so as to form a slot which will just pass a quarter-inch thumb or wing-nut bolt. These uprights form the optical bed along which the other parts of the apparatus may be clamped in any position by means of winged nuts (fig. 1, *w*). The lamp house (fig. 1, *h*) consists of a small mazda lantern-slide projector apparatus without the lantern-slide carrier or the projecting lens. This lamp house carries a 400-watt concentrated filament mazda projection bulb backed by a concave mirror which gives a strong steady light. The lamp house is fastened to the optical bed by means of two wing-nuts which pass through a board (fig. 1, *b*) to which the lamp house is securely bolted. The microscope (fig. 1, *m*) is fastened in a similar manner, but it is necessary to have the microscope block (fig. 1, *m b*) much thicker, so that the optical axes of the light and the microscope will center. The microscope is securely fastened to the micro-

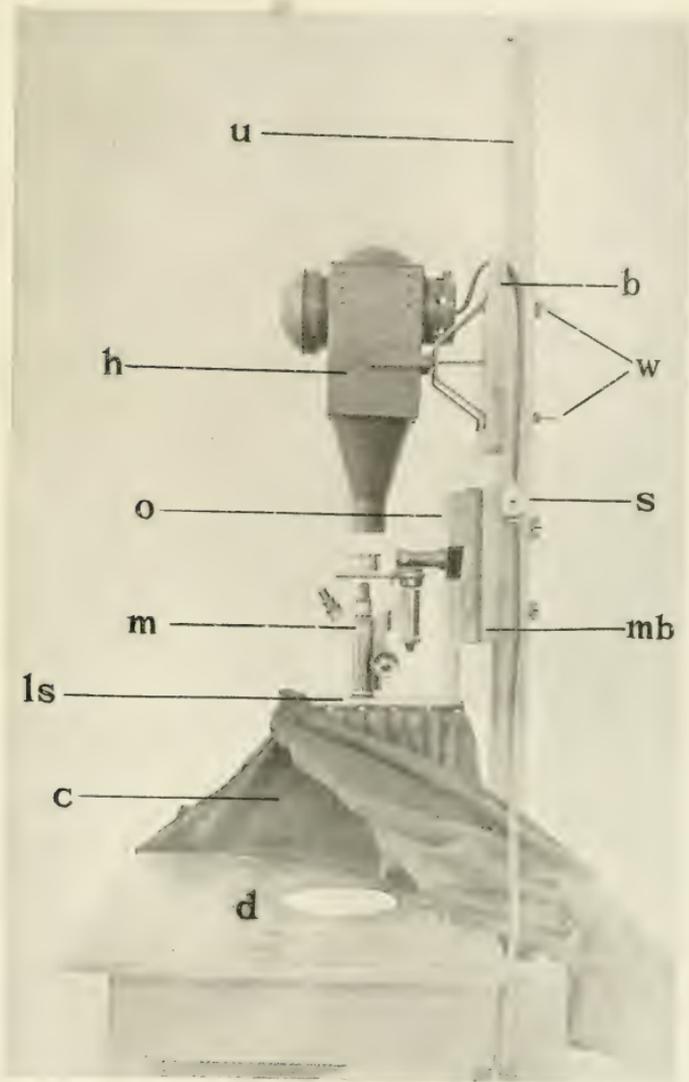


Figure 1

scope block by means of blocks which are slotted to receive the prongs of the horseshoe base of the microscope. The base is held securely by a piece of strap-iron (fig. 1, *o*), which is held in place by means of two screws. We used an old discarded microscope for this purpose, but it would be very simple to fasten the strap-iron by means of wing-nuts, in that case any microscope could be clamped in place for use and then unclamped for laboratory use. The lower part of the microscope block is projected and has a light shield (fig. 1, *l s*) fastened at right angles. This light shield is slotted to receive the tube of the microscope and has a dark curtain (fig. 1, *c*) tacked to its edges, so that the apparatus can be used in a lighted room.

The cost of the apparatus exclusive of the microscope was as follows:

1 lamp house with 400-watt mazda lamp.....	\$32.00
1 table.....	5.00
1 drawing board.....	1.25
Lumber for optical beds, etc.....	.50
1 dozen bolts.....	.10
$\frac{3}{4}$ yard black broadcloth.....	2.25
Total.....	\$41.10

A DUPLICATE SCALE

Having a great many bilaterally symmetrical drawings to make, we had a special scale made as illustrated (fig. 2). This scale is 30 cm. long and reads from the center toward both ends. One edge is graduated in millimeters and has every centimeter numbered. The other edge is graduated in centimeters only. The scale is used in the following manner: A central line is drawn along the central axis of the drawing and one-half of the drawing outlined by means of the camera lucida or by any other method. The scale is then laid on the drawing with its center on the central axis of the drawing. It is a very simple matter to move the scale along the axis and to read the location of critical points on the outline which has been drawn and to plot them on the other end of the scale.

A simple illustration, such as the outline of the beetle here presented, will make the matter clear. *A B* is the central axis

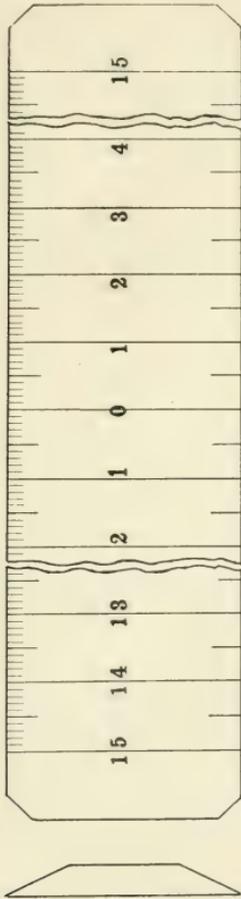


Figure 2

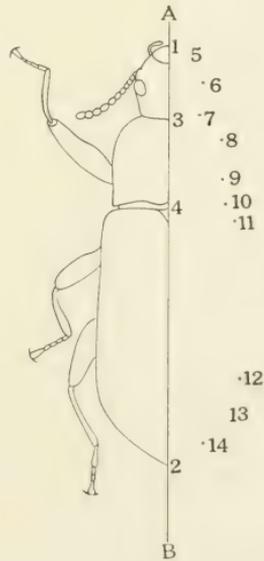


Figure 3

of the drawing and the left side of the outline has been secured. The problem is to secure the outline of the right side. The scale is centered on the axis *A B* and moved along until the

base of the clypeus is reached. It is noted that this point is 8 mm. from the central axis, and a point (fig. 3, *point 5*) on the right-hand side of the axis is located, the same number of millimeters from the central axis. In the same way the scale is moved along and other critical points are located (fig. 3, *points 6 to 14*). The number of points necessary will depend upon the complexity of the outline and will be in inverse ratio to the skill of the draughtsman. I have found this a very useful scale and far superior to transferring by carbon paper or other methods that I have tried, especially when working on wash or water-color drawings where the number of marks on the paper should be reduced to the minimum.

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The Author can greatly assist the Publishers of this Journal in attaining prompt publication of his paper by following these three suggestions:

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Resumen por G. Carl Huber, por el autor Juan C. Nañagas.

Sobre la patencia del orificio oval en los niños filipinos recién nacidos.

La serie estudiada por el autor comprende 134 casos, 80 varones y 54 hembras, la mayor parte de los cuales habían nacido muertos (117) presentando el aspecto normal del feto a término. Las observaciones sobre el foramen oval se relacionaron con la longitud y peso, tamaño y peso del corazón y espesor de la pared cardiaca. El orificio oval existía bajo tres aspectos diferentes: su forma era circular, oval o triangular; la primera era la más pequeña y más frecuente, y el último tipo el mayor y menos frecuente. El autor no ha podido observar relación definida alguna entre el tamaño del corazón y el del orificio oval, ni tampoco entre el tamaño del orificio y la abertura de la patencia.

Translation by José F. Nonidez
Cornell Medical College, New York

ON THE PATENCY OF THE FORAMEN OVALE IN FILIPINO NEWBORN CHILDREN

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THREE FIGURES

The scarcity of reports on the patency of the foramen ovale in the anatomical literature, specially from this country, and the desire of perhaps throwing some light on the etiology of the abnormally high rate of infant mortality in these islands have induced us to make some observations on the hearts of newborn babies coming to our morgue from the different hospitals and out-patient clinics of Manila.

The present work is offered only as a preliminary note, and as such it is incomplete and based on a small number of cases. It is intended to have it followed later by more extensive observations.

MATERIAL AND TECHNIQUE

Our series includes 134 cases, 80 males and 54 females, the majority of which were stillborn and outwardly at least apparently normal and full-term foetuses (117 stillbirths, fourteen under twelve hours and three under six days).

We are only considering the morphology, position, and different degrees of patency of the foramen ovale, but in order to establish the stage of development of the foetus and of its heart we have taken in addition the measurements of body length, body weight, size of the heart and of its different orifices, cardiac weight, and the thickness of cardiac walls.

After determining the body weight and the body length in each case, the heart was removed by cutting the great vessels uniformly at the level of the superior atrial borders. It was then weighed fresh with the contained blood unremoved and with calipers, the greatest vertical, transverse, and antero-

posterior diameters were determined. The difficulties in keeping the parts in situ while measuring and the consequent distortions following the opening of the cardiac cavities have decided us to fix the organ in formalin (10 per cent) before undertaking any internal examinations. About a month later, the heart was again weighed, its greatest diameters once more taken and in addition the greatest circumference of its base was determined.

The heart was now opened by four different incisions:

1. A vertical incision was made along its dorsal wall extending from the superior caval orifice down to the inferior, thence downward to the apex of the right ventricle, cutting through the right atrioventricular orifice. We expose by this incision the right side of the interatrial septum and the right atrioventricular orifice.

2. A second incision was cut along the ventral wall of the right ventricle from the lower end of the preceding, and extending upward to the pulmonary orifice in a direction parallel to the interventricular septum. We expose for examination the pulmonary orifice.

3. A third incision extending from the upper left pulmonary vein along the posterior wall of the heart downward to the bottom of the left ventricle, passing through the mitral opening. We lay open by this cut the left side of the interatrial septum.

4. A fourth and last incision was then made from the cut end of the aorta along the posterior wall of the vessel, the medial wall of the left artium and down to the cavity of the left ventricle, passing through right anterior flap of the mitral valve. We expose here the aortic and mitral orifices.

We measured the thickness of the cardiac walls and the cardiac diameters with calipers, the cardiac circumference with a graduated tape, and the areas of the foramen ovale and of its patent opening with a graduated slide specially devised for the purpose.

We etched on a glass slide an area of 1 sq. cm. which was divided into 100 sq. mm. Below the large square a linear scale divided in tenths of millimeters was etched. This slide was placed as near as possible to the surface to be measured and as nearly parallel to its plane; the number of squares included within the field were counted, partial squares being compensatingly considered, i.e., two halves equal to one, etc.

GENERAL BODY AND CARDIAC MEASUREMENTS

We note from Benestad and Meyer the following figures for the average body weights, body lengths, and cardiac weights of newly born children, to which we have added our own findings:

TABLE I
Different measurements of body lengths, body weights, and cardiac weights

AUTHOR	PLACE	NUMBER OF CASES	MALE	FEMALE	BOTH SEXES
<i>a. Average body weights</i>					
			<i>grams</i>	<i>grams</i>	<i>grams</i>
Issmer.....	Dresden	7612	3320	3214	3267
Wernick.....	Munich	6000	3337	3209	3273
Ingerslev.....	Copenhagen	3450	3381	3280	3330
Kerzmarszky.....	Jena	1700	3484	3344	3414
Paterson.....	Upsala	1675	3595	3455	3525
Wagner.....	Konigsberg	1500	3479	3339	3409
Wolff.....	Basel	1448	3220	3140	3180
C. Martin.....	Berlin	1000	3328	3221	3274
Fuhrmann.....	Petrograd	1000	3490	3185	3337
Bonestad.....	Copenhagen	1979	3528	3410	3469
Meyer.....	United States	1026	3387	3242	3436 ¹
Meyer.....	United States	1027			3185 ²
Katzen.....	Toulouse	1000	3305	3175	3240
Biedert.....			3500	3250	3375
Nañagas.....	Manila	134	2253.7	2251.7	2252.7 ³
<i>b. Average body lengths</i>					
			<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
Katsen.....	Toulouse	1000	49.9	48.9	49.4
Meyer.....	United States	1026	49.6	48.7	49.8
Meyer.....	United States	1027 ¹			48.5
Biedert.....					50.0
Nañagas.....	Manila	134	44.2	44.0	44.1
<i>c. Average cardiac weights</i>					
			<i>grams</i>	<i>grams</i>	<i>grams</i>
Muller.....			16.2	14.4	
Vierordt.....			17.2	15.2	
Nañagas.....			15.5	15.3	
Bovaire and Nicoll.....					21.0
Notori.....					14.5
Nañagas.....					15.4

¹ Negroes. ² White. ³ Stillborn.

Our own general maximum, minimum and average measurements were as follow:

TABLE 2
*Body weights, body lengths, and cardiac weights*¹

SEX	BODY WEIGHTS			BODY LENGTHS			CARDIAC WEIGHTS		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
Males.....	4630	815	2253	57.0	21.0	44.2	26.0	6.9	15.5
Females.....	3844	850	2251	58.0	21.0	44.0	24.1	6.0	15.3
Average, regardless of sex.....	4237	832	2252	57.5	21.0	44.1	25.0	6.4	15.4

¹ Weights in grams and lengths in centimeters.

The cardiac weights were averaged from the results in fresh and fixed hearts. For the purpose of establishing the relations between body weights, body lengths, and cardiac weights, we prepared table 3, where the cases were grouped in series varying in weights of 200 grams increase, the measurements representing the averages for the number of cases included in each series.

For a comparative study, we also reproduce here from Scammon the measurements of weights of the heart in the newborn.

Table 3 of Scammon's collection of foetal measurements

BODY WEIGHT	HEART WEIGHT
<i>kpm.</i>	<i>grams</i>
2.0 to 2.5	17.8
2.5 to 3.0	19.5
3.0 to 3.5	21.7
3.5 to 4.0	25.3
4.0 to 5.0	28.6

From table 3 we note that the average cardiac weights bear definite relations with the average body weights, such relations with one or two exceptions consisting in a steady proportionate increase of about 1.13 grams for each 200 grams of increase in body weight. Comparing our results with those given in Scam-

mon's table, it will be noted that the Filipino hearts were correspondingly lighter, the difference varying from 1.42 to 2.50 grams.

Our measurements of the dimensions of the heart are shown in table 4, in which the cases were segregated in groups of increasing cardiac weights.

TABLE 3
Relations between body weight, body length, and cardiac weight

NUMBER OF CASES	BODY WEIGHTS	AVERAGE OF BODY LENGTH	HEART WEIGHTS		
			Minimum	Maximum	Average
	<i>grams</i>	<i>cm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
7	Under 1000	36.0	5.2	12.5	6.67
7	1000-1200	40.0	7.7	15.3	10.00
9	1200-1400	38.5	6.8	21.0	8.84
13	1400-1600	41.5	7.7	12.0	9.60
13	1600-1800	42.3	9.0	15.5	11.11
9	1800-2000	44.0	10.1	15.0	11.92
7	2000-2200	42.5	7.1	19.6	14.30
13	2200-2400	47.1	10.3	24.0	15.68
11	2400-2600	46.3	11.2	24.0	15.83
11	2600-2800	48.0	7.2	26.0	16.42
12	2800-3000	47.6	15.0	21.0	16.93
6	3000-3200	50.2	16.0	20.4	18.60
7	3200-3400	50.4	18.3	25.0	20.90
7	3400-3600	52.2	14.4	41.0	21.27
2	Over 3600	53.0	18.7	19.9	19.30

Our general averages of the above cardiac dimensions were:

Greatest circumference.....	92.1
Greatest vertical diameter.....	46.2
Greatest transverse diameter.....	34.9
Greatest anteroposterior diameter.....	22.4

Unfortunately, we find no record of measurements of the heart in the newborn. Barthes and Rillet, quoted by von Stark, give some measurements in children of fifteen months to fourteen and one-half years. Their cardiac circumference varied from 130 to 190 mm. and the vertical diameter from 55 to 90 mm.

From table 4 we find that hearts weighing from 5 to 20 grams showed a consistent increase in all dimensions, which became

TABLE 4
Cardiac dimensions in relation to cardiac weights

HEART WEIGHT	NUMBER OF CASES	CIRCUMFERENCE	DIAMETERS		
			Vertical	Transverse	Anteroposterior
<i>grams</i>					
5-10	29	72.6	35.7	27.5	18.2
10-15	46	86.3	42.9	31.9	21.0
15-20	32	97.1	45.7	36.5	22.8
20-25	13	100.0	48.9	37.8	26.1
25-30	5	104.5	58.0	41.2	24.0

less marked in the heavier hearts. This apparent discrepancy between the increase in weight and the lessened increase in dimensions is probably to be accounted for by a greater increase in the thickness of cardiac walls.

With reference to the parietal thickness of the heart, we found the following:

TABLE 5
Comparative measurements of thickness of the ventricular walls

AUTHOR	AGE	THICKNESS OF VENTRICULAR WALLS	
		Right	Left
		<i>mm.</i>	<i>mm.</i>
Gibson and Gillespie.....	Full-term (s.b.)	4.0	3
Gibson and Gillespie.....	Full-term (lived 24 hrs.)	3.0	4
Notori.....	(Newborn)	4.0	4
Nañagas.....	(Stillborn)	3.6	4

Our average measurements of the circumference of the other cardiac orifices were:

	<i>mm.</i>
Right atrioventricular orifice.....	27.5
Left atrioventricular orifice.....	25.2
Pulmonary orifice.....	15.9
Aortic orifice.....	13.6

Table 6 shows the measurements of the cardiac orifices and cardiac walls in relation to cardiac weights.

The left atrioventricular, the aortic, and the pulmonary orifices showed a steady and proportionate increase in size with the increase cardiac weights, the right atrioventricular opening, however, appeared to remain stationary in hearts weighing 20 grams or more.

The thickness of the two ventricular walls increased about the same at the rate of 5 mm. for every 5 grams of excess in cardiac weight. The wall of the left ventricle was thicker than the right throughout the series, the difference being greater in lighter hearts.

TABLE 6

Measurements of cardiac walls, cardiac orifices and cardiac weights

HEART WEIGHTS	NUMBER OF CASES	THICKNESS OF VENTRICLE		CIRCUMFERENCE OF CARDIAC ORIFICE			
		Right ventricle	Left ventricle	Right atrio-ventricular orifice	Left atrio-ventricular orifice	Pulmonary orifice	Aortic orifice
<i>grams</i>		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
5-10	30	26.0	32.0	23.5	21.5	12.8	11.5
10-15	47	35.0	37.0	27.0	24.5	15.0	13.0
15-20	31	39.9	43.5	30.0	26.5	17.0	14.5
20-25	12	45.0	48.0	29.5	28.5	19.0	15.5

Revising the above data, we find that the average body weight and body length in our series were less than those given by other observers. In our mind this fact, however, cannot be construed as spelling lack of development and immaturity of our foetuses. The probabilities are that the measurements of the other authors were taken on the living in some obstetrical clinics, while ours were made on dead subjects and only after forty-eight hours of steady refrigeration. Moreover, it is a well-known fact that Filipinos are a small race and, as noted elsewhere, the foetuses were outwardly normal in appearance.

The cardiac weights though correspondingly smaller, as compared to those given by Scammon, yet our general average appears almost normal when compared with those of Muller, Vierordt, Notori, and others. We are unfortunate in not finding records of measurements of cardiac dimensions in the literature,

yet if we compare our average figures with those of Barthes and Rillet for babies fifteen months old, the discrepancy appears to be only what might be expected.

The measurements of the thickness of the ventricular walls were almost identical with the findings of Gibson and Gillespie and Notori, though in our series the left ventricle showed a trifle excess in thickness as compared to the right.

We may safely conclude, therefore, that our foetuses as well as their hearts were not far from their normal development.

TABLE 7
Frequency of the three types of foramen ovale

SEX	TOTAL NUMBER OF CASES	CIRCULARS		OVALS		TRIANGULARS	
		Cases	Percentage	Cases	Percentage	Cases	Percentage
Male.....	63	28	44.4	22	34.9	13	20.7
Female.....	44	27	61.3	9	20.4	8	19.3
Total.....	107	55	53.4	31	27.6	21	20.0

FORAMEN OVALE

The foramen ovale was studied in 107 cases. It showed considerable variation in shape, however; we could distinguish three principal types distributed as follows (table 7 and figs. 1, 2, and 3).

a. Circular foramen, present in 55 cases (28 males and 27 females), or in 53.4 per cent of the series. It is more frequently encountered in females. The posterior border was the least prominent, the other margins of the foramen merging gradually with the posterior atrial wall at this point.

b. Ovale type, found in 31 cases (22 males and 9 females), or in 27.6 per cent. The long axis of the foramen was directed anteroposteriorly and the superior and inferior margins were much more prominent than the edges in front and behind. This type was found more in males.

c. Triangular form, present in 21 cases (13 males and 8 females), or in 20 per cent, and with equal frequency in both



sexes. The foramen had one of its angles directed anteriorly and the two posteriorly. The sides of the anterior angle were better developed than those of the posterior angles.

TABLE 8
Areas of foramen ovale (in sq. mm.)

CIRCULARS				OVALS				TRIANGULARS			
Males		Females		Males		Females		Males		Females	
Cases	Area	Cases	Area	Cases	Area	Cases	Area	Cases	Area	Cases	Area
1	6	1	5	1	10	1	17	1	12	1	13
1	12	1	9	1	13	1	19	2	14	1	14
1	13	1	11	2	16	1	20	1	15	1	18
1	14	1	12	1	19	1	22	1	17	1	24
1	16	2	13	1	23	1	25	1	19	1	26
2	17	1	14	1	25	1	28	1	24	1	37
1	19	2	16	1	27	1	32	1	25	1	39
2	20	3	17	2	28	1	44	1	26	1	95
1	21	1	19	1	29	1	45	1	27		
1	22	2	20	1	33			1	28		
1	23	1	21	2	36			1	36		
1	24	3	23	1	38			1	54		
2	26	1	24	1	39						
2	27	1	26	1	42						
1	29	1	28	3	43						
2	31	2	30	1	52						
2	34	1	37	1	85						
1	36	1	46								
1	39	1	56								
1	41										
1	42										
1	50										
28	25.6	27	21.77	22	35.18	9	18	13	23.9	8	33.2

The foramen ovale was uniformly found located in the posterior two-thirds of the interatrial septum, sometimes at its middle (in 66 cases, or 61.4 per cent), others higher up near the superior caval opening (in 39 cases, or 36.4 per cent), and in 13 cases (or 12 per cent) in its lower section near the valve of Thebesius. The areas of the foramen are given in table 8.

The general average in the total series was 26.69 sq. mm. From the above table it will be seen that the circular type showed the smallest area, averaging only 23.69 sq. mm.; the oval form came next, with a general average of 26.59 sq. mm., and the triangular shaped foramina appeared largest, with an average of 28.55 sq. mm. Both the circular and oval types showed larger average areas in males than in females, while in the triangular forms the conditions were reversed. In general we found that most of the cases varied in area between 20 and 30 sq. mm.

TABLE 9
Areas of patency of foramen ovale (sq. mm.)

CIRCULAR				OVAL				TRIANGULAR			
Males		Females		Males		Females		Males		Females	
Cases	Area	Cases	Area	Cases	Area	Cases	Area	Cases	Area	Cases	Area
2	3.0	1	1.5	3	2.0	3	2.0	1	1.0	2	3
1	4.0	5	2.0	2	2.5	1	3.0	1	1.5	1	18.0
2	5.0	1	2.5	1	3.0	1	4.0	3	3.0		
1	6.0	2	3.0	2	3.0	1	5.0	2	4.0		
5	7.0	1	4.0	4	4.0	1	6.0	1	6.0		
1	8.0	1	5.0	1	5.0						
1	26.0			2	6.0						
				1	27.0						
13	7.33	11	2.09	16	5.0	7	3.42	8	3.18	3	38.0

PATENCY OF THE FORAMEN OVALE

Addison found in his series 33.3 per cent of patent cases. We encountered a much greater number of cases in ours. Out of 107 cases examined, 58 showed patency, or 54.2 per cent; 36 of these were met among males (57.1 per cent) and 22 cases were found in females (64.7 per cent).

The above cases were distributed in the different types of foramen ovale as shown in table 9.

We found patency in 24 out of 55 circular cases (42.7 per cent); in 23 out of 31 oval cases (74.1 per cent), and in 11 out of 21 triangular cases (52.3 per cent). The area of the aperture

varied from 1 to 27 sq. mm., the largest being found in males of the oval and circular types. Most of our cases varied in area from 2 to 5 sq. mm.

The position of the aperture of patency appears to vary with the position of the septa of the foramen, so that the opening is pushed upward when the septum primum has its free edge directed also superiorly and vice versa when the conditions are reversed. The edge of the septum secundum apparently has a contrary effect in directing the location of the opening, i.e., if the free edge of the septum primum is directed upward it pushes ahead the aperture, while the edge of the septum secundum meeting it from above tends to shift ahead of it the opening, and between the two of them they reduce its area of extent. In over one-half of the cases the opening appeared above the middle horizontal plane bisecting the foramen ovale. This is only to be expected from the most common position of the two septa: the septum primum, postero-inferiorly and the septum secundum, anterosuperiorly. This same position of the septa accounts for the oval shape of the aperture and the oblique direction of its long axis from above and behind forward and inferiorly. Physiologically, this orientation of the opening appears favorable in directing the blood stream from the inferior caval orifice to the left atrium.

In 78 cases the septum primum was posterior in position, while the septum secundum was anterior; in 42 cases the septum primum was postero-inferior, while the septum secundum was anterosuperior in 34 cases, and finally the septum primum was posterosuperior in one case, and the septum secundum antero-inferior in 8 instances.

Reviewing the tables giving the sizes of the heart, and the areas of the foramen ovale and its aperture of patency, we failed to find any definite relation between the size of the heart and that of the foramen, or between the area of the foramen ovale and its orifice of patency.

SUMMARY

Summarizing our findings:

1. Filipino foetuses in general are smaller and lighter than European or American foetuses.

2. The general average weights of foetal hearts in our series was about the same as those reported by other observers. Cardiac weights increased proportionately with body weight, this increase was, however, smaller than given in Scammon's table 33.

3. Cardiac dimensions showed a definite and proportionate increase with the increase in cardiac weights; this increase, however, was less marked in hearts heavier than 20 grams, probably because of greater thickening of cardiac walls.

4. The thickness of the two ventricular walls in our cases, was about the same as reported by other workers, but in our series the left ventricle was uniformly the thicker.

5. The thickness of both ventricles definitely and proportionately increased with the cardiac weights.

6. The right atrioventricular orifice showed the largest circumference; the aortic, the smallest. The left atrioventricular, the aortic, and the pulmonary openings steadily increased in size with the weights of the hearts, but the right atrioventricular orifice remained almost stationary in hearts heavier than 20 grams.

7. Filipino foetuses and foetal hearts of this series were probably normal in development.

8. The foramen ovale was present in three different shapes: circular, oval, and triangular; the first of which was the smallest and most frequently encountered, and the last type the largest and least common.

9. The circular and oval types of foramen ovale were larger in males than in females, while in the triangular form the conditions were reversed. The circular type was more commonly encountered in females, while the other two types were more frequent in males.

10. In location the foramen ovale was most commonly found in the middle section of the posterior two-thirds of the interatrial septum.

11. Our percentage of patency of the foramen ovale was greater than reported by Addison. More cases of patency were met in females.

12. The area of the opening of patency was largest in males. It varied from 1 to 27 sq. mm., the majority of cases were 2 to 5 sq. mm.

13. In position the opening was more commonly found above the middle horizontal plane of the foramen ovale.

14. No definite relation could be observed between the sizes of the hearts and of the foramen ovale, nor between the size of the foramen ovale and the aperture of patency.

We feel that we are not in possession of sufficient data to offer conclusions explanatory of the high frequency of occurrence of patent foramen ovale in Filipinos; we believe, however, that this fact should be taken into account when considering the various and different etiological factors of our excessive infant mortality.

Our thanks are due to Dr. Arturo Garcia for helpful suggestions in the present work.

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Resumen por G. Carl Huber, por el autor Sabas E. Yap.

El músculo esternal de los filipinos.

En 136 cadáveres adultos diseccionados, el autor ha observado la presencia de un músculo esternal en cinco casos, en cuatro mórstruos anencefálicos entre diez examinados, y en dos fetos entre diez fetos normales estudiados. Los casos observados se describen detenidamente, ilustrando las descripciones con figuras. Las variaciones relativas al origen é inserción, relaciones, y la inervación, encontrados en los casos descritos y en otros publicados en la literatura han sido reunidos en una serie de cuadros. El autor presta particular atención a la homología del músculo esternal, encontrando diversas opiniones en la literatura, las cuales discute críticamente, llegando a la conclusión de que este músculo pertenece al grupo de los músculos pectorales—una opinión primeramente expresada por Bardeleben y apoyada más tarde por Cunningham y Shepherd por el hecho de que el músculo esternal está a menudo inervado por los nervios torácicos esternales.

Translation by José F. Nonidez
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MUSCULUS STERNALIS IN FILIPINOS¹

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TWO PLATES (TEN FIGURES)

The musculus sternalis is a small muscle of the thorax encountered in the dissection-room in no less than 3 per cent of all cases. For a long time it has aroused the interest of many anatomists because of the different possibilities of explaining its homologies. It was described for the first time in 1604 by Cabrolus (1), but its connections and relations were not precisely understood until 1723, when Du Puy gave a clear account of the muscle. From that time on this muscle has been very extensively discussed, and in recent years its homology has taxed the ingenuity of many investigators, and various interesting hypotheses had been formulated in their attempts to explain it. Its varied nerve supply, connections, and relations which have been considered as important points in deciding its homology make of this muscle an interesting specimen to anatomists.

It has been found in both sexes in adult cadavers and in a surprisingly large proportion in anencephalous foetuses. It has been reported in different races. Its occurrence in Filipinos is the subject of this short paper.

The present work comprises an observation on 136 cadavers dissected in the Department of Anatomy for the last five years, and includes besides a special dissection made on ten anencephalous monsters (four males and six females) and ten apparently normal foetuses (five males and five females).

I was able to collect data of its presence in five out of the 136 adult cadavers, or a proportion of about 3.6 per cent, and found it present in four anencephalous monsters of the ten examined (40 per cent) and in two of the normal foetuses (20 per cent).

¹ Read before the Manila Medical Society, October 4, 1920.

Two of the five adult cadavers showing the muscle were males, and in each one it was present on only one side; the remaining three were females, and in each the muscle was bilateral. We also found the muscle bilateral in the four monsters which were all females and in one male and one female normal foetus. We therefore had a total of twenty specimens. Attention is invited to the fact that most of our dissected adult cadavers were males.

The following are the cases observed:

Case I. Male, 36 years old, prisoner. *Musculus sternalis*, unilateral, left side. It measured 2 cm. at its widest part and about 10 cm. in length. It originated by a flattened tendinous band from the pars abdominalis of the left pectoralis major at the level of the sixth rib about 3.75 cm. from the center of the xiphoid process of the sternum.

Its fibers ran upward and medially and gained attachment by a small tendon to the second costal cartilage, blending there with the sternal origins of the pectoralis major and the sternocleido mastoids from the manubrium sterni. The nerve supply was furnished by fibers from the medial anterior thoracic which penetrated the deep surface of the muscle near its medial margin.

Case II. Female, 85 years of age, *Musculus sternalis*, bilateral. Both muscles were about the same in length and width, 10 cm. long and 2.5 cm. wide. Each originated from the pectoralis major at the level of the fourth rib, the fibers ran upward and medially and were inserted to the anterior surface of the sternoclavicular joint, blending with the sternal origin of the corresponding sternocleido mastoid muscle. The nerve supply was also from the anterior thoracic nerves.

Case III. Female, insane, 89 years old, *Musculus sternalis*, bilateral. The left muscle was shorter and much smaller than the right. It originated by a tendinous band from the left rectus sheath and the sixth rib, the fibers ran upward and medially to be inserted by tendinous band into the manubrium sterni at the level of the second rib. A tendinous slip separated from this tendon of insertion and crossed medially to unite with the tendon of the right *Musculus sternalis*. The nerve supply was from the anterior thoracic.

The right muscle originated also from the right rectus sheath and sixth rib by a tendinous band, and ran upward and medially to gain insertion in common with the sternal origins of the two sternocleido mastoid muscles. The nerve supply was identical to that of the left muscle.

Case IV. Female, insane, 78 years old, *Musculus sternalis*, bilateral. Both muscles were of about the same size (fleshy portions about 4 cm. long and 1.5 mm. wide) each originating by a long tendinous slip from the fifth costal cartilage and by radiating muscular fibers from the aponeuroses of the external oblique muscle of the abdomen. The course of the muscle fibers was similar to that of the others, upward and medially

and were inserted to the junction of the manubrium and the body of the sternum. The nerve supply was not determined. (We were not able to make a drawing of this case.)

Case V. Male, prisoner, 20 years old, musculus sternalis, unilateral, right. It measured 10 cm. long and 2.5 cm. wide. It originated from the fascia covering the lower portion of the right pectoralis major, it ran upward and medially and was inserted by two tendinous slips, the upper one to the middle of the lower part of the manubrium sterni at the level of the second rib, and the lower slip to the middle of the body of the sternum at the level of the third rib. The nerve supply was from one of the intercostals.

Case VI. Female normal foetus. Musculus sternalis, bilateral. The right muscle was 4.4 cm. long and 0.8 cm. wide (widest part). It originated by muscular fibers from the sixth cartilage and the costal origin of the pectoralis major. The fibers coursed upward and medially gradually tapering to a slender muscular band which was continuous with the right sternomastoid. The nerve supply was a twig from the lateral anterior thoracic. The medial border of the origin was about 0.6 cm. from the midsternal line.

The left was 4 cm. long and 0.95 cm. wide (widest part). Origin by a wide and flat tendinous band from the aponeurosis of the external oblique, the rectus sheath, and costal portion of the pectoralis major. It ran upward and medially and was partly inserted by muscular fibers to the sternal portion of the pectoralis major at the level of the second interspace and partly by muscular fibers which crossed the middle line of the sternum at the level of the second rib to be inserted into the right pectoralis major. The nerve supply not identified. The medial border of the origin was 1.1 cm. from the midsternal line.

Case VII. Male normal foetus. Musculus sternalis bilateral. The right muscle was 3.4 cm. long by 0.5 cm. wide at widest point. Origin, by aponeurotic band from the costal portion of the pectoralis major at the level of the fifth interspace, the fibers course upward and medially and at the level of the third interspace, the muscle bifurcated, the lateral muscular fasciculus was inserted to the sternal portion of the great pectoral at the level of the third rib, and the medial band which was semitendinous was continuous with the sternomastoids above. The nerve supply was not found. The medial border of the origin was 0.9 cm. from the midsternal line.

The left was 3.7 cm. long and 0.9 cm. wide (widest part). It originated by muscular fibers from the sixth rib and the adjoining costal portion of the great pectoral, the fibers course upward and medially, tapering to a semitendinous band which also become continuous with both sternomastoids. The nerve to the muscle was not found. The medial border of the origin was 0.95 cm. from the middle line.

Case VIII. Anencephalous foetus, female. Musculus sternalis, bilateral. The right muscle was much larger than the left, it measured 4.5 cm. long and 1.7 cm. wide. It originated by a tendinous band partly from the aponeurosis of the external oblique and partly from the

pars abdominalis of the right pectoralis major. The medial border of its origin was 1.2 cm. from the middle line. The fleshy fibers ran upward and medially, making a curve convex outward and gradually tapering to be inserted by muscular fibers to the middle of the lower portion of the manubrium sterni and by deep fibers to the second costal cartilage. There was a distinct triangular interval between this muscle and the clavicular and abdominal portions of the pectoralis major, where its nerve was seen coursing to penetrate the substance of its deep surface. By reflecting laterally the upper portion of the clavicular and sternal portions of the pectoralis major, this nerve was traced over the pectoralis minor from the musculus sternalis upward and laterally to the point where it pierced the costocoracoid membrane, where it was identified as the medial anterior thoracic nerve.

The left muscle originated by a flat thin tendon not unlike ordinary fascia from the lower part of the costal portion of the left pectoralis major, it ran upward and medially, and at the level of the second rib, it was joined at its lateral side by a small muscular slip from the left pectoralis major. It divided at this level into two flat tendons, the lateral one was inserted with the right muscle to the middle of the lower portion of the manubrium sterni and the medial tendon to the middle of the upper portion of the body of the sternum. This muscle measured 2.7 cm. long and 0.4 cm. wide. The medial border of its origin was 0.5 cm. from the middle line. No nerve to this muscle was observed.

Case IX. Anencephalous foetus, female. Musculus sternalis, bilateral. The right muscle was smaller than the left. It measured 2 cm. long and 0.7 cm. wide. Its origin was by muscular fibers from the pars abdominalis of the right pectoralis major, some of the fibers originating from its deep surface. The muscle ran upward and medially and was inserted by fascial attachment to the sternal portion of the right pectoralis major at the level of the third rib. The medial border of its origin was 1.2 cm. from the middle line. Its nerve supply pierced both pectoral muscles and reached the musculus sternalis at its deep surface. It was identified to be the medial anterior thoracic.

The left muscle was much larger, it measured 3.5 cm. long and 1.1 cm. wide. Its origin was by a flat tendinous band from the aponeurosis of the external oblique, it ran upward and medially and was inserted by two tendinous ends, the lateral to the third costal cartilage and the medial crossed the median line to the right side and was inserted in common with that of the right muscle to the middle sternal portion of the right pectoralis major at the level of the third rib. The medial border of its origin was about 1.4 cm. from the middle line. Its nerve supply was identical with that of the right muscle.

Case X. Anencephalous monster, female. Musculus sternalis, bilateral. The right muscle was much larger than the left measuring 5.4 cm. long and 1.7 cm. at its widest part. The medial border of the origin was 1.8 cm. from the midsternal line. It originated from rectus sheath and aponeurosis of the external oblique, the fibers ran upward and medially, and at the level of the third costal cartilage it bifurcated, the lateral

muscular band was inserted into the sternal portion of the pectoralis major at the second interspace and the medial end tapered in a tendinous band inserted into the sternal portions of the two pectorals at about the median line. The nerve supply was by a filament from the third intercostal.

The left was very small, it measured 2.1 cm. long and 0.3 cm. at widest portion. Its origin was muscular from the costal portion of the great pectoral at the level of the fifth rib 1.1 cm. from the median line of the sternum, the fibers ran upward and medially and were inserted directly to the sternal portion of the pectoral at the level of the upper border of the third rib. Nerve supply was not found.

The right muscle was larger. It measured 4.4 cm. long and 1 cm. wide. It was apparently made up of two parts, separated by denser fascia, one superficial and lower originated from the aponeurosis of the external oblique and the abdominal portion of the great pectoral, its fibers ran upward and medially, and at the level of the fourth costal cartilage they blended with those of the deeper part and continued upward to gain insertion by semitendinous connection to the manubrium sterni. The deeper portion originated by muscular slips from the aponeurosis of the external oblique and by tendinous origin from the costal portion of the great pectoral under cover of the superficial part, and after pursuing a vertically upward course was inserted by muscular attachment to the anterior part of the sternoclavicular joint. The medial border of the origin of the muscle was 1.2 cm. from the median line. Its nerve was not found.

The left was much smaller. It measured 2.2 cm. long and 0.25 cm. wide. Origin, by long slender tendinous slip from the sternal portion of the pectoralis major at the level of the fourth costal cartilage, the fibers ran upward and medially and were inserted directly to the upper border of the manubrium sterni near the median line. Nerve supply not found. The medial border of the origin was 0.4 cm. from the midsternal line.

Table 1 shows the distribution of the muscle.

From the above cases we see that the attachments, relations, and sizes of the musculus sternalis are so manifold that a description of one specimen is not an adequate account for all, no two specimens being exactly alike. In this respect it is different from other muscles of the body, which, as a rule, have a constant origin, insertion, relations, and nerve supply. Its size in our cases varied from 2 cm. to 10 cm. long and from 0.25 cm. to 2.5 cm. wide. We can say, however, that in every case, the muscle was found lying over the pectoralis major at varying distances from the sternum. With regard to origin we found that the muscle

started sometimes from the aponeurosis of the external oblique, at others from the abdominal portion of the great pectoral, rectus sheath, or from the lower costal cartilages. Again this origin varied from fascial bands to muscular or tendinous slips.

Its insertions again were varied. In some cases it was found inserted in the manubrium sterni, in others in the body of the

TABLE 1

SCHOOL YEAR	NUMBER OF SPECIMENS EXAMINED	PRESENCE OF MUSCULUS STERNALIS						INNERVATION
		Cases	Male	Female	Unilateral or bilateral	Number of specimens	Percentage	
<i>a. Musculus sternalis in adult Filipino cadavers</i>								
1915-16	60	I	1		Unilateral	1	1.66	Medial anterior thoracic
1917-18	38	II and III		2	Bilateral	4	5.26	Both anterior thoracic
1918-19	14	IV		1	Bilateral	2	7.14	Undertermined
1920-21	24	V	1		Unilateral	1	4.16	Intercostal
Total	136	5	2	3		8	3.67	
<i>b. Musculus sternalis in Filipino foetuses</i>								
1920	10	VI and VII	1	1	Bilateral	4	20	Lateral anterior thoracic
<i>c. Musculus sternalis in anencephalic Filipino foetuses</i>								
1920	10	VIII to XI		4	Bilateral	8	40	Medial anterior thoracic and third intercostal

sternum and upper costal cartilages, or even blending with the upper origins of the pectoralis major or the sternal origins of the sternocleido mastoids. Here also the insertion was accomplished either by muscular fasciculi or by one or more tendinous connections. The nerve supply is by no means the same in every case. In our cases, however, two nerves appeared to be the main sources of nerve supply, viz., the anterior thoracics and the intercostals. This finding is confirmed by those of others, as noted in table 2.

The importance of the nerve supply in considering the homology of this muscle was not apparently realized by the older writers, and it is only since Cunningham (2) called attention to this fact that more careful study has been made of this part of the subject.

TABLE 2

Nerve supply of the musculus sternalis (modified after Cunningham)

AUTHORS	NUMBER OF MUSCLES SUPPLIED BY ANTERIOR THORACICS	NUMBER OF MUSCLES SUPPLIED BY INTERCOSTALS
Shepherd.....	7	
Wallace.....	1	
Lamont.....	6	
Dwight.....	2	2
Cunningham.....	17	
Hallett.....		1
Bardeleben.....		2
Yap.....	9	2
Total.....	42	7

TABLE 3

Musculus sternalis in different nationalities (modified after Ruge)

AUTHORS	NATIONALITIES	PERCENTAGE
Chudzinski, Le Double and Testut.....	Negro	8.4
Macalister.....	Irish	6.0
Cunningham.....	Irish	4.4
Gruber.....	Russian	5.26
Le Double.....	French	4.65
Wood.....	English	4.0
Turner.....	Scotts	3.23
Schwalbe and Pfitzner.....	Alsace-Lorraine	3.24
Yap.....	Filipinos (adults)	3.67

In the beginning of this paper I called attention to the fact that the musculus sternalis was found in different nationalities. I am inserting the following table showing this occurrence and its relative proportion in different races as reported by various writers (table 3).

The occurrence of this muscle in the two sexes is variable. In adults some authors found it more commonly in males, while others met it more frequently in females, and there are those who reported it with equal frequency in the two sexes. I found it present in adult females in the proportion of 3 to 2, in normal foetuses 1 to 1, and all the cases encountered in anencephalic foetuses were in females.

TABLE 4

Relative frequency of musculus sternalis in the two sexes (modified after Cunningham)

AUTHORS	MALE	FEMALE
Bardeleben's table.....	27	25
Bardeleben.....		2
Malbranc.....	2	
Shepherd.....	3	
Le Double.....	2	1
M. Issaurat fils.....	1	
Joessel.....	1	
Dwight.....	3	3
Curnow.....	2	3
Cunningham.....	8	8
Wallace.....		1
Lamont.....		4
Turner.....	7	11
Ingalls.....	1	
Yap ¹	2	3
Total.....	59	61

¹ Only adult cases given.

Hallett (2) regards this muscle as inspiratory in function. Cunningham claims that if this were the case we should expect to find it present in greater proportion in females and his statistics show forty-nine males and forty-seven females, but he failed to include Turner's findings, on the ground that more females were encountered in the latter's dissecting-room. Without intending to argue the matter, we wish to call attention to our table 4 and to our previous statement regarding the preponderance of male cadavers in our dissecting-room. Moreover, we found the

muscle present in five female foetuses and in only one male, and Shepherd in five out of six female anencephalous monsters.

As to its occurrence as a double muscle in the two sexes, the same uncertainty is noted. I found it bilateral in all my female cases, while in all the male cases, except the male normal foetus, it was unilateral.

TABLE 5

Relative frequency of double sternalis in the two sexes (modified after Cunningham)

AUTHORS	MALE	FEMALE
Bardeleben's table.....	6	4
Melbranc.....	1	
Le Double.....	1	1
Joessel.....	1	
Dwight.....	1	1
Curnow.....	1	2
Cunningham.....	2	2
Ingalls.....	1	
Yap ¹		3
Total.....	14	13

¹ Only adult cases given.

In the anencephalous foetuses it was noted in much greater proportions, varying from 16 to 88 per cent. I found it present in my series in about 40 per cent. There is as yet no definite explanation advanced to account for this frequency of occurrence. Shepherd (4) seems to believe that it points rather to its being a rudiment than a new muscle:

Windle (cited by Ruge (3)) comes forward with the suggestion that the muscle might be found in greater proportion in lunatics, evidently basing his belief on the frequency of its presence in anencephalous monsters. He does not, however, attempt to offer any basic reason for such suggestion. We cannot at present advance any opinion on this aspect of the subject; we wish, nevertheless, to call attention to the high percentage of the musculus sternalis (40 per cent) in the anencephalous foetuses examined by us, and the fact that out of five adults which showed the muscle in our series, two were chronic insanes and two were

criminals. It would seem that this point might be further looked into with a view of explaining the connection between this anomaly and insanity, if there is really such a relation.

TABLE 6
Musculus sternalis in anencephalic fetuses (modified after Ruge)

AUTHORS	NUMBER OF CASES	NUMBER OF TIMES FOUND	PERCENTAGE
Shepherd.....	9	8	88.0
Eisler.....	7	4	57.0
Abraham.....	11	6	54.0
Windle.....	27	10	37.0
Cunningham.....	6	1	16.6
Yap.....	10	4 ¹	40.0
Total.....	70	33	47.14

¹ Double.

The homology of the musculus sternalis is as yet a question that remains unsettled. Anatomists who have given extensive accounts of the muscle vary in their views. Some based their conclusions chiefly from its connections, while others took into consideration its nerve supply, and there are those who look upon it as a muscle sui generis and peculiar to man. There are at present five hypotheses of interest regarding the homology of this muscle:

1. That the muscle is a remnant of the paniculus carnosus and associated in man with the platysma myoides.

This theory is ably and extensively discussed by Professor Turner (1), whose views are shared by Hallet, Parson, Lambert (3), and a few others.

Turner seems to base his conclusions on these observations:

a. That in muscular subjects the platysma myoides descended over the clavicle and above the pectoralis major for a considerable distance (2 to 4 inches) on the anterior surface of the chest.

b. The fact that occasionally portions of the paniculus carnosus, besides the platysma and the palmeris longus, are found in other parts of the body.

c. The occasional crossing of the platysma myoides in front of the sternum as observed by Teichmann, resembling in such cases the position assumed sometimes by the musculus sternalis.

d. That in lower animals the paniculus carnosus is well developed in the ventral surface, and extends with a marked process over the pectoralis major.

There are two points which in our mind speak against the above hypothesis:

a. The fact that the platysma is found in the superficial fascia, whereas the musculus sternalis is under it, so that the two muscles are in two distinct morphological planes.

b. And the other, as Cunningham (2) points out, is the innervation of the muscle. He correctly reasons that if the muscle were a part of the paniculus carnosus, we should expect it to be innervated by a special branch from the brachial plexus, whereas all the observations reported so far indicate that it derives its nerve supply from the anterior thoracic nerves or the intercostals.

Parson (3), in his work on rodents, claims that the musculus sternalis may be the persistence of the deeper layer of the pectoral section of the paniculus carnosus. But here again the nerve supply of the muscle speaks against this view.

2. Albinus (1) first advanced a theory which regards the musculus sternalis as an upward extension of the rectus abdominis muscle. Turner, however, claims that in all those mammals where the rectus abdominis is prolonged upward, the fibers of the prolongation were always encountered under the great pectoral muscle and in contact with the ribs, whereas the musculus sternalis in man is always found superficial to the pectoralis major.

3. Bourienne (3) is responsible for the theory which regards the musculus sternalis as a downward prolongation of the sternocleido mastoid muscle. He was later supported by Henle, Marjolin, Gegenbaur, Theile (3), and others. It is a point of common observation that the sternal origin of the sternomastoid is in common with the insertion of the musculus sternalis. I found this to be the case in five out of eleven cases and Turner in thirteen out of twenty one. Cunningham (2), in discussing this view, says that if the musculus sternalis were a part of the

sternomastoid, we should expect it to be innervated by some branch of the spinal accessory or the cervical plexus, which occurrence, however, has never been reported. Ruge (3) claims that in no animals the sternomastoid extends to the abdomen or to the aponeurosis of the external oblique, and that, furthermore, the innervation of the musculus sternalis is against the above theory.

As modifications of the above two hypotheses we also find the following views:

a. Testut with Anthony (3) believes that the upper part of the musculus sternalis belongs to the sternomastoid, while its lower portion is a part of the external oblique muscle. This contention is based upon the claim that these two muscles (sternomastoid and external oblique) are in the same morphological plane and "that the musculus sternalis is the remnant in man of the old connection which formerly existed between the two, a connection which exists in serpents." Cunningham (2), however, contends that such a connection becomes the pectoralis major in the thrusting out of limbs. Besides, we must remember that serpents are not philogenetically related to mammals.

b. Bardeleben (2) claimed that the musculus sternalis when receiving its nerve supply from the intercostals must be considered as belonging to the sternomastoid, and when innervated by the anterior thoracics to the pectoral group.

Cunningham (2) takes exception to this view, on the ground that in the majority of cases which he recorded as supplied by the anterior thoracic nerves, the muscles were directly continuous with the sternomastoid. This fact was confirmed by us in five of our cases; furthermore, there are cases in which musculus sternalis, though innervated by the intercostals, were not continuous with the sternomastoid.

4. That the musculus sternalis is a muscle sui generis and peculiar to man, without a representative in lower animals. This view was advanced by Professor Halbertsma (1).

5. The musculus sternalis belongs to the pectoral group of muscles. This last view was first advanced by Bardeleben, who claimed that a certain proportion of the muscles could be considered as portions of the pectoralis major.

Abraham (2) applied this view more extensively. He considered all sternal muscles without exception as aberrant portions of the great pectoral.

Cunningham and Shepherd, on the strength of the innervation of the muscle by the anterior thoracic nerves, strongly support the above view. Their finding has been confirmed by other observers and we have also verified it in six cases.

The above authors further contend in favor of this theory the fact that some fibers of the musculus sternalis in some instances appeared directly continuous with the pectoralis major. Such instance was encountered several times in our series.

Another point which might also be mentioned in support of this hypothesis is the presence of a gap or deficiency in the fibers of the great pectoral at the lateral side of the musculus sternalis.

Such occurrence was reported by Shepherd in four of his cases and we have met it once in our study. We agree with Cunningham when he said, "it is reasonable to suppose that the gap is caused by the abstraction of this portion of the muscle to form the sternalis and there are many cases figured which would lead one to suspect that the rotation of fibres has taken place in an upward and inward direction."

The above explanation would surely account for those cases where the lower end of the muscle lies under cover of the great pectoral. Ruge, after an extensive discussion of the above different theories is inclined to agree with Cunningham that the sternalis is probably a portion of the pectoralis major.

Our present study inclines us to agree with the preceding explanation of the homology of the muscle. We have found the muscle supplied by the anterior thoracic nerves in six out of eight cases; we met it in close relationship with the pectoralis major and in several cases there was a direct muscular connection between the two, either in the points of origin or at the ends of insertion, and finally we also found one case where a decided gap was seen in the pectoralis major just lateral to the sternalis.

Furthermore, we quote the following from Lewis (6) on the formation of the pectoralis major: "The pectoralis major early splits into a series of overlapping bundles, and during the migra-

tion of the muscle the superficial fibres of each bundle move farther caudally than the deeper ones, giving the overlapping condition found in the adult."

It will not be too far fetched to suppose that sometimes some of the superficial fibers split from the pectoral muscle mass and fail to migrate caudally with the rest, forming in that manner the narrow sternalis muscle.

We wish to express our indebtedness to Dr. Otto Schobl, of the Bureau of Science, for kindly helping us in the translation of some of the literature.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

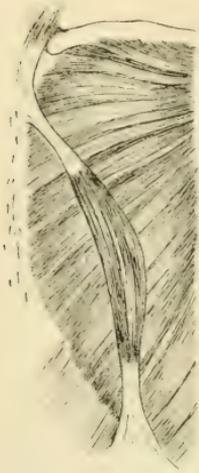
Drawings by Vicente Santos

Case I Male, 36 years. *Musculus sternalis* unilateral, left. Origin: Pars abdominalis of left pectoralis major at level of sixth rib. Insertion: Second costal cartilage blending with pectoralis major and sternomastoid. Nerve supply: Medial anterior thoracic.

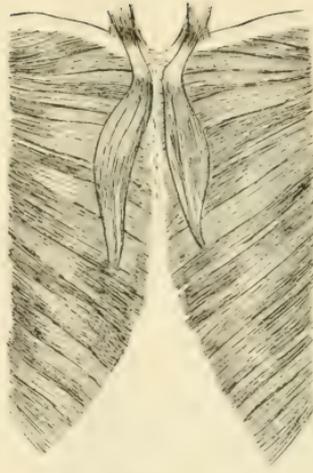
Case II Female, 85 years. *Musculus sternalis* bilateral. Origin: (Both) pectoralis major at level of fourth rib. Insertion: Anterior surface of sternoclavicular joint blending with corresponding sternomastoid. Nerve supply: Anterior thoracic.

Case III Female, 89 years. *Musculus sternalis* bilateral. Left muscle, shorter and smaller. Origin: Left rectus sheath and sixth rib. Insertion: Manubrium sterni at level of second rib. A tendinous slip from this unites with the tendon of the right muscle. Nerve supply: Anterior thoracic. Right muscle: Origin: Right rectus sheath and sixth rib. Insertion: In common with the sternal origins of the two sternomastoid. Nerve supply: Anterior thoracic.

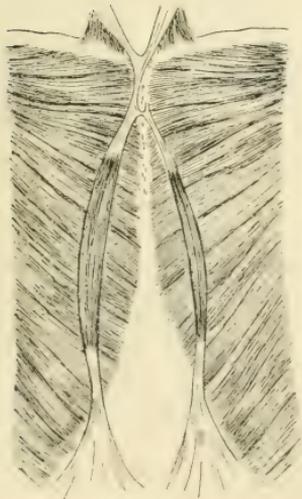
Case V Male, 20 years. *Musculus sternalis*, unilateral, right. Origin: Fascia covering lower portion of pectoralis major. Insertion: Upper tendon to manubrium sterni; lower tendon to body of sternum at level of third rib. Nerve supply: Intercostals.



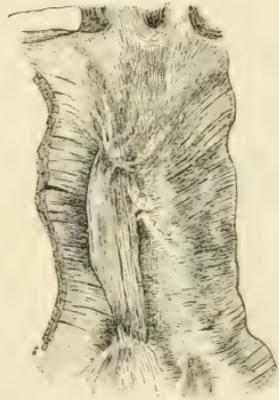
Case I



Case II



Case III



Case V

PLATE 2

EXPLANATION OF FIGURES

Drawings by Vicente Santos

Case VI Normal foetus, female. *Musculus sternalis*, bilateral. Right muscle: Origin: Sixth costal cartilage and costal origin of *pectoralis major*. Insertion: Continuous with the right *sternomastoid*. Nerve supply: Lateral anterior thoracic nerve. Left muscle: Origin: Aponeurosis of external oblique, rectus sheath, and costal portion of *pectoralis major*. Insertion: Sternal portion of *pectoralis major* at level of second interspace and to the right *pectoral major* at level of second rib. Nerve supply: Not identified.

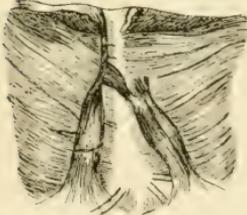
Case VII Normal foetus, male. *Musculus sternalis*, bilateral. Right muscle: Origin: Costal portion of *pectoralis major* at level of fifth interspace. Insertion: Sternal portion of great pectoral at level of third rib and to the *sternomastoids*. Nerve supply: Not found. Left muscle: Origin: Sixth rib and adjoining portion of the *pectoralis major*. Insertion: To both mastoid muscles. Nerve supply: Not found.

Case VIII Anencephalous monster, female. *Musculus sternalis*, bilateral. Right muscle: Origin, aponeurosis of external oblique muscle and pars abdominalis of the great pectoral. Insertion: Lower portion of manubrium and to second cartilage. Nerve supply: Medial anterior thoracic. Left muscle: Origin: Costal portion of *pectoralis major* and by a small slip from the middle part of the muscle. Insertion: Lower portion of manubrium and upper portion of the body of the sternum. Nerve supply: Not found.

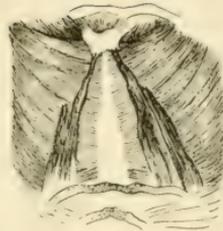
Case IX Anencephalous monster, female. *Musculus sternalis*, bilateral. Right muscle: Origin: Pars abdominalis of great pectoral; Insertion: Sternal portion of right pectoral major at level of third rib. Nerve supply: Medial anterior thoracic. Left muscle: Origin: Aponeurosis of external oblique. Insertion: Third costal cartilage and in common with the right *musculus sternalis* into the sternal part of the great pectoral muscle at level of third rib. Nerve supply: Medial anterior thoracic.

Case X Anencephalous monster, female. *Musculus sternalis*, bilateral. Right muscle: Origin: Rectus sheath and aponeurosis of external oblique. Insertion: Sternal portion of great pectoral at level of second interspace and into sternal portions of the two pectorals at median line. Nerve supply: Third intercostal. Left muscle: Origin: Costal portion of great pectoral at level of fifth rib. Insertion: Sternal portion of great pectoral at level of upper border of third rib. Nerve supply: Not found.

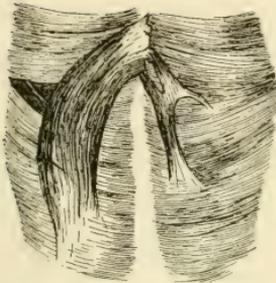
Case XI Anencephalous monster, female. *Musculus sternalis*, bilateral. Right muscle: Origin: Aponeurosis of external oblique and abdominal portion of great pectoral. Insertion: Manubrium sterni and anterior part of the sternoclavicular joint. Nerve supply: Not found. Left muscle: Origin: Sternal portion of great pectoral at level of fourth costal cartilage. Insertion: Upper border of the manubrium sterni. Nerve supply: Not found.



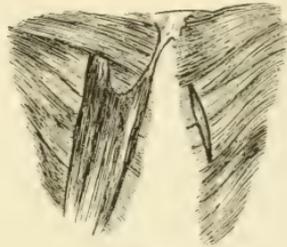
Case VI



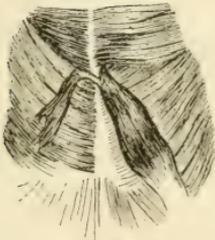
Case VII



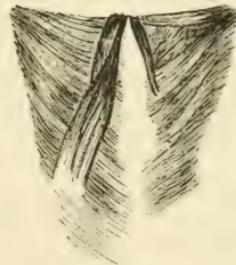
Case. VIII



Case X



Case IX



Case XI

Resumen por el autor, Charles W. Metz.

Un sencillo método para manejar objetos pequeños durante la obtención de preparaciones microscópicas.

Para preparar objetos muy pequeños con el fin de obtener cortes, el autor ha encontrado muy útil el empleo de la piel que rodea el abdomen de las ninfas de la mosca de los frutos, *Drosophila*. Separando la cubierta ninfal, para lo cual se separará el abdomen del resto de la ninfa, y comprimiendo dicha cubierta se obtiene un pequeño saco transparente en el cual pueden introducirse los objetos que se desea preparar. Este saco puede llenarse durante la fijación o antes de depositar los objetos en el alcohol de 70. Se puede obtener un gran número de ninfas exponiendo al aire frutas fermentadas, y las cubiertas pueden guardarse hasta el momento de usarlas en alcohol de 30 o 50 grados. Si se emplean ninfas de varias especies pueden obtenerse cubiertas de diversos tamaños.

Translation by José F. Nonidez
Cornell Medical College, New York

A SIMPLE METHOD FOR HANDLING SMALL OBJECTS IN MAKING MICROSCOPIC PREPARATIONS

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In the course of cytological studies on flies, especially *Drosophila* and other small forms, I have met with considerable difficulty in transferring the very small gonads during the processes of fixing, washing, dehydrating and embedding. After trying various methods of handling these objects the following was worked out and has proven so satisfactory that I am led to make a brief record of its essential features.

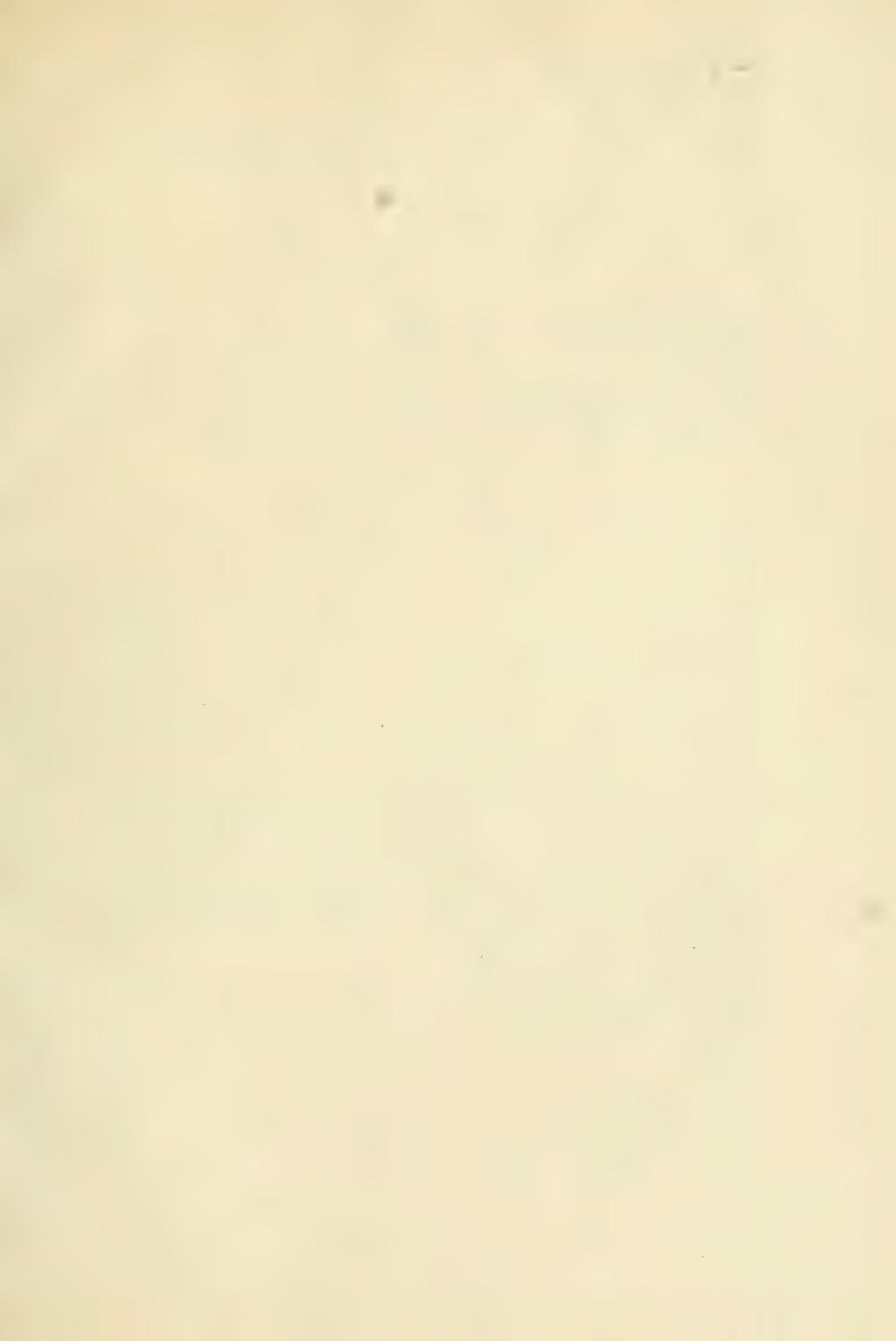
The method is similar to the well known one of wrapping the objects in *Cryptobranchus* epidermis or some other transparent membrane; but instead of a flat sheet of tissue it utilizes the bag-like skin surrounding the abdomen of the *Drosophila* (fruit fly, vinegar fly, banana fly) pupa. At about the middle of the pupal stage the abdomen of a *Drosophila* is covered with a thin, transparent skin, under the chitinous pupa case. The skin may be obtained intact by cutting the pupa in two across the thorax, removing the posterior part from the case and pressing out the contents. A simple procedure for this is to puncture the pupa with a needle about a third of the way back from the anterior end (end with the long 'horns'), then transfer it to water and tear it in two with dissecting needles, after which the abdomen and the attached portion of the thorax may easily be pulled or pressed from the pupa case and their contents removed by gentle pressure with a needle. The latter is facilitated if the apex of the abdomen is held down with the point of the other needle.

An oval, transparent bag remains, having a somewhat constricted opening at the anterior end representing the connection between abdomen and thorax. The small objects to be treated may be passed through this into the pouch until the latter is

nearly full and then the opening may be closed by pressing or twisting the edges together with needles or forceps and immersion in the fixative or alcohol. I have usually fixed and washed the objects and run them up to 50 per cent alcohol before packing them into the pupa-skins. This obviates the necessity of leaving them long in the dissecting fluid during the dissecting and packing into the pouch. But with other objects such as small eggs, etc., which do not involve much dissection, or when such exposure to the dissecting fluid is not injurious, the packing may be done before fixation.

Once a packet is made in this manner and the membrane has hardened in the alcohol or fixative it may be handled readily without fear of losing the contents. Reagents, including paraffin, penetrate the membrane readily, so that dehydrating and embedding may be done rapidly. Since it is easy to secure species of *Drosophila* of various sizes around garbage, decaying fruit, etc., it is possible to obtain pupa-skins of sizes adaptable to individual needs. By putting a few flies in a cotton-plugged bottle with ripe banana (or something similar) and a little paper, practically any desired number of pupae may soon be obtained. The pupa-skins may be prepared as noted above and kept in 30 per cent or 50 per cent alcohol until needed. Thus a supply sufficient for months or years may be made up at one time.

At first this method seems somewhat tedious, but with a little practice it proves to be rapid and efficient. Among its favorable features may be mentioned, 1) standardization of size of packets, 2) convenience of size of packets, 3) minimum amount of enclosing tissue together with close aggregation of objects within, making it unnecessary to section a packet much larger than the mass of objects themselves—a considerable saving—4) transparency of the enclosing membrane at all stages, permitting orientation or examination of the contents, 5) cleanliness of membrane and freedom from dirt or grit such as is often encountered in amphibian epidermis, and 6) relative freedom from extraneous tissue or material in the sections, thus facilitating their examination.



Resumen por el autor, Lee D. Cady.

Un estudio microscópico del fascículo senoventricular del corazón del conejo, con referencia a los datos relativos a su interpretación funcional, especialmente en términos de un manantial de reemplazo del miocardio degenerado.

El nodo del seno y el nodo atrioventricular son prácticamente idénticos en estructura histológica en el conejo. En estos nodos puede observarse la presencia de numerosos núcleos en varios estados de división amitótica, pero los núcleos de las células binucleadas están colocados muy próximos entre sí y no existe prueba de la división protoplásmica. La porción principal del fascículo atrioventricular presenta una estructura muy semejante. En las fibras de Purkinje, sin embargo, los núcleos están más separados que en las porciones del sistema conductor situado más encima. En las áreas de transición entre las fibras de Purkinje y el miocardio ventricular, se encuentran tan solo núcleos impares. En el tejido nodal o en el tejido del fascículo no existe indicio alguno que indique la división citoplásmica de las células, ni tampoco hay dato histológico que indique un reemplazamiento de las fibras miocárdicas degeneradas por una progresión genética de las células a partir del nodo del seno hacia el miocardio ventricular.

Translation by José P. Nonidez
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A MICROSCOPICAL STUDY OF THE SINOVENTRICULAR BUNDLE OF THE RABBIT'S HEART; WITH REFERENCE TO THE DATA RELATIVE TO ITS FUNCTIONAL INTERPRETATION, ESPECIALLY IN TERMS OF A SOURCE OF REPLACEMENT OF DEGENERATED MYOCARDIUM

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ONE PLATE (FIVE FIGURES)

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INTRODUCTION

This study of the sinoventricular bundle was undertaken with the view of testing Retzer's ('20) recent rather startling conclusion that worn out myocardium is replaced from the sinus region of the heart, the new myocardial fibers being derived from the Purkinje fibers, which in turn are said to be derived from higher regions of the sinoventricular system. He compares the sinus region to the basal layer of cells in stratified (transitional) epithelium in that both of them are differentiated in the embryo, and he thinks that the sinus cells continue to multiply in a manner analogous to the manner in which the basal cells of stratified epithelium multiply in adult life. In 1908 Retzer expressed his belief that the Purkinje fibers are derived from the sinus tissue, but said nothing about a possible derivation of myocardial fibers from the Purkinje fibers. Retzer's conclusion is based for the most part upon a study of the pig's heart.

That there is a direct transition between the Purkinje fibers and the myocardial fibers was shown by Tawara ('06), and confirmed by Retzer ('08), Lhamon ('12), DeWitt ('09), and others, who traced the Purkinje fibers to their transition into cardiac fibers; but none mentioned indications of a possible replacement of degenerated myocardial fibers from that source.

This paper is concerned with the study of the rabbit's heart only with reference to the sinoventricular bundle, as Retzer terms the entire conduction system from sinus to Purkinje fibers, inclusive, and purports to present the data as found in the material studied with reference to its functional interpretation, especially as regards the replenishment of worn-out myocardial fibers by the sinoventricular bundle. No attempt was made to study the 'right lateral connection' of the atrioventricular junction, as Kent ('13) calls it, for its functional existence has apparently not been substantiated (Erlanger, '08). Retzer ('08) describes it in the pig's heart. We have been unable to find any really clear clinical demonstration that such a connection exists, and the experiments of Erlanger and Blackman ('10) show conclusively that there is either no such connection or that, if there is such a connection, it does not function as a pathway of conduction when there is greatest physiological need for it, namely, when the sinoventricular system is destroyed.

MATERIALS AND METHODS

The heart of the rabbit shows the conductive system with extraordinary detail in routine preparations from some of the specimens, there being some variation in this respect. About twenty rabbits' hearts were studied. They varied in age from one week to the well-matured specimens that can be found in the ordinary animal room. Sections were also kindly furnished by Dr. H. E. Jordan, which were invaluable in carrying out this work. Altogether something over three thousand sections were examined.

Details regarding technique were taken from Jordan's 'Text Book of Histology' ('20). A variety of fixing methods were used, including Flemming's, Meves', Zimmermann's, Zenker's,

Zenker-formol, 95 per cent alcohol, and 10 per cent formalin. One adult heart was opened by a longitudinal incision through the lateral wall of the right atrium and the cut continued down through the ventricle until the apex was reached. The parts of the lateral walls of both atrium and ventricle were then pulled aside and the whole heart flattened with thin strips of cork against a clean glass slide, weighted down in that condition with light weights, and covered with Zenker-formol fixing fluid. By this method it was possible to include in the longitudinal sections of the heart longer sections of the conductive system. A one-week-old heart, fixed in 95 per cent alcohol, was sectioned transversely; i.e., at right angles to the long axis of the heart. The sections were mounted serially and stained with hematoxylin and eosin. The atrial septum of a young adult, likewise fixed in 95 per cent alcohol, was sectioned longitudinally and mounted serially, beginning with the endocardial surface of the left atrium, and stained with hematoxylin and eosin. These sections were designed to show the nodal tissue. Blocks of the septum and of the walls of both ventricles were sectioned longitudinally, in order to get longitudinal and oblique sections of the transition areas of the Purkinje fibers. The material furnished by Doctor Jordan, a celloidin preparation, fixed in Zenker's fluid, was cut transversely, and stained with hematoxylin and eosin.

All serial sections were mounted five or six to the slide, and the sections on alternate slides were stained with hematoxylin and eosin, while the remaining sections were stained either with iron hematoxylin and picro-fuchsin or by other special methods, including Mallory's phosphotungstic acid-hematoxylin. Blocks of tissue from two hearts were stained with hemalum after Zimmermann fixation for intercalated discs. Silver impregnation methods were also used. All sections were mounted in Canada balsam.

DESCRIPTION

Keith and Flack's ('06) description of the sinus node in the human heart agrees closely with what is found in the rabbit's heart. They describe the nodal fibers as striated, of fusiform shape, with well-marked elongated nuclei, plexiform in arrangement, and imbedded in densely packed connective tissue—in fact, of a structure very like that of the atrioventricular node. There is slightly more connective tissue in the atrioventricular node in our sections than in the sinus node (figs. 1 and 2), but this tissue is not abundant enough to warrant the description as 'closely packed.' The nuclei could hardly be described as 'well-marked elongated nuclei,' for they are more nearly oval. A careful comparative study of the two nodes in the sections fails to reveal any distinct difference other than those already mentioned. They further state that these two nodes resemble each other in other mammalian hearts, the resemblance being especially marked in the mole, rat, and the ram. To this enumeration we can add that they are practically identical in appearance in the rabbit.

In the atrioventricular node (fig. 2) and the sinus node (fig. 1) the cross-striations of the fibers appear only very faintly in some of the hearts. In others the striation is more conspicuous. The myofibrils are peripherally placed; the nuclei are located centrally and they are surrounded by a relatively large amount of undifferentiated sarcous material which is rather granular. Frequently, however, in the regions of the nuclei there is no evidence of granules in the stained sections. Only rarely can one find any fibrils immediately adjacent to the nuclei. The nuclei are large, oval, and rather vesicular in appearance. They contain scattered granules of chromatin material which, except for a few chromatin clumps, is pretty evenly distributed. Many of the nuclei show one or two nucleoli. Frequently one finds two nuclei, rather smaller and more heavily stained than the larger single nuclei within the same perinuclear spaces and in close juxtaposition. No evidence of mitotic division can be seen in the cells of either of these nodes. Connective-tissue cells are scattered rather sparsely throughout the sectioned surfaces of

either node, but slightly more connective tissue is in evidence in the atrioventricular node. There seems to be no definite connective-tissue sheath surrounding either node. The nodal fibers of the sinus pass by gradual transition into the atrial fibers and are soon lost in them. The two nodes resemble each other so closely that one is safe in saying that they are identical in appearance, for, unless one knows the exact region of the section, it is impossible to identify either node with any degree of certainty except by the relative amount of connective tissue, which is in itself a variable (Cohn, '09).

The cells of the sinoventricular bundle below the region of the sinus node, that is, in the atrioventricular node, have very well-defined boundaries (fig. 3). The cytoplasm in stained sections appears pale, and in many of the cells shows only slight differentiation. The cell peripheries stain more deeply than the remainder of the cytoplasm, and at the levels of the nuclei show peripherally placed myofibrils, which are finer than those of the myocardial fibers. The nuclei are usually situated in relatively large clear areas and in the central portion of the cells. In fact, the large clear central area is the most conspicuous feature of these cells. The nuclei are rather pale, but well stained. Chromatin granules are fine and pretty evenly distributed, but nuclei are easily found that show chromatin clumps as in the atrioventricular node. Well-defined nucleoli, which take a pink stain in the hematoxylin and eosin preparations, are found in the nuclei; indeed, many nuclei show from two to four nucleoli. A large percentage of the cells have two nucleoli.

One cell was noticed that appeared to have four nuclei within the same perinuclear space. Where there are two nuclei, they are usually somewhat smaller than the nuclei in the mononucleated cells and they are darker in appearance. The binucleated cells also have nuclei that closely resemble the single nuclei in size, shape, and staining reaction. This type of nucleus is generally less closely apposed to its fellow than are the smaller and darker nuclei, which are usually somewhat flattened against each other. Nuclei in the various stages of amitotic division are frequently seen. However, no cells were seen that had their

nuclei widely separated or showed evidence of cytoplasmic division. No mitotic figures were seen in any portion of the system. The bundle is separated from the ventricular myocardium by means of a thin connective-tissue sheath. Connective-tissue cells are found also scattered throughout the entire bundle. The bundle has a subendocardial position everywhere except in the moderator band and in the so-called 'false tendons.'

In the case of the moderator band and of the false tendons (fig 3) the above description of the bundle also applies except that these particular subdivisions of the sinoventricular bundle are still further subdivided by connective-tissue septa, delicate in the moderator band, relatively robust in the false tendons, where the larger strands of the bundle are enveloped by relatively dense connective-tissue sheaths.

The Purkinje fibers (figs. 4 and 5) are only slightly more differentiated than the bundle cells. The nuclei are of about the same size and appearance as those of the bundle except at the points of transition into the myocardial fibers where they are smaller and gradually pass over into the myocardial type of nuclei. As the transition areas are approached, the binucleated cells become progressively fewer until the transition area is reached, where only unpaired nuclei are found. The cytoplasmic transition is more gradual than that of the nuclei, and it is almost impossible to determine just where Purkinje fibers actually end and the myocardial fibers begin. The Purkinje fibers are larger than the myocardial fibers, but at the transition points they diminish in diameter gradually and take on a more conspicuously striated and fibrillated condition. The connective-tissue sheath of the Purkinje fibers is very delicate and often does not show, especially at the transition points.

DISCUSSION

To answer the question whether the sinoventricular bundle consists of embryonal tissue and whether it may be able to differentiate into myocardium, one must consider its histogenesis. The cells of the sinoventricular bundle are differentiated early in embryonic life (eleven weeks in Keith's human embryo). The

facts that their myofibrils are more delicate and more peripherally placed and less conspicuously striated than in the myocardium and that there is more undifferentiated sarcous material centrally in these cells do not argue conclusively that they are embryonic cells. Their large size, their variously stout fusiform, polyhedral, and irregular shapes, and their striated fibrillar structure, all jointly indicate that they are specialized cells. Lange ('14) arrives at the conclusion also that the Purkinje fibers are not embryonic remains, since they are clearly distinguishable in very young mammalian hearts. He concludes further that they are a non-nervous apparatus for conducting the impulse to heart beat. The most suggestive evidence in favor of Retzer's conclusion relates to his observation that in the cells of the terminal portions of the bundle the nuclei have moved relatively farther apart. But this fact may be equally plausibly interpreted as the result of a secondary (mechanical) elongation of the cells of this region consequent to the imposition of stresses during the growth of the heart.

Howell ('19) states that it is uncertain whether the atrio-ventricular bundle contracts during systole, but that there is little doubt as to its function of conduction of the impulse to heart beat. Intercalated discs are not found in the atrio-ventricular bundle, but they are present in the Purkinje fibers (Jordan, Banks, '17). They occur very early in fetal life in the myocardium, during the second month in the heart of the beef (Jordan, Banks, '17). Assuming that intercalated discs are modified irreversible contraction bands produced through functional strains and stresses, we come to the conclusion that the atrioventricular bundle either does not contract, or at least, if it does contract, it does not share the strains ordinarily incident to heart beat, and that the Purkinje fibers do contract. However, the cells of the bundle show cross-striation, which is in itself an indication of their muscular nature. Curran's ('09) conclusion is that the presence of the constant bursa (interpreted by Lhamon ('12) as a connective-tissue sheath and not as a bursa) which he describes surrounding the atrioventricular bundle indicates that this muscle bundle either does not contract at all or that it con-

tracts in a different way and at a time which is not synchronous with the contraction of the ventricular myocardium, and he inclines to the latter view. His observation on a beating heart lead him to believe that the atrioventricular bundle contracted synchronously with the atria, but his observation was not confirmed by graphic methods. Erlanger ('12) was not able to demonstrate any contraction in excised segments of false tendons even when they were perfused through their own blood vessels with various solutions. The fact that there are no intercalated discs in the atrioventricular bundle does not prove that it does not contract, nor does the fact that there are cross-striated myofibrils in the bundle prove that it does contract. Embryonic hearts, and tissue cultures from embryonic chick hearts, exhibit rhythmical contraction before intercalated discs or the full complement of cross-striations appear. Cross-striations are apparently not necessary for contractility in heart muscle. Doctor Burrows kindly made it possible for us to examine some of his tissue-culture preparations of embryonic chick hearts ('12). We found no intercalated discs nor cross-striations, but only irregularly arranged myofibrils within the sarcoplasm. These preparations were known to have shown rhythmical contraction for relatively long periods of time. It may be possible that the sinoventricular bundle conducts the impulse to heart beat and still does not itself contract. If the calcium be removed from one end of a strip of myocardium and that end be stimulated, it will conduct the impulse for contraction to the untreated end, which immediately contracts, but the treated end will not contract. A knowledge of the calcium content of the bundle would be interesting in this connection. However, the only conclusive method of determining whether or not the bundle tissue contracts is to study its activity, if any, by improved graphic physiological methods.

If Retzer's unique conclusion that the myocardial fibers are replenished from the sinoventricular bundle is correct, it is very difficult to reconcile this interpretation with the facts disclosed by pathological and experimental data. Keith and Miller ('06) reported a case of syphilis of the heart where the superior

vena-cava region of the atrium, the coronary sinus, and the atrioventricular node had been destroyed about thirteen years before death. ". . . the muscular connection between auricle and ventricle had been destroyed several years (probably 13) before death, yet the complete section of the bundle had not resulted in any visible atrophy beyond the point of destruction. Were this peculiar system, . . . only a conducting pathway for the auricular stimuli into the ventricle, one would expect degeneration in the system of fibers beyond the point of section, for it may be taken as an axiom in biology that abrogation of function is invariably followed by atrophy." This heart showed no signs of failure or patent symptoms of cardiac insufficiency once the patient had recovered from the symptoms incident to the onset of his bradycardia (about forty-two beats per minute) until his death twelve years later as a result of acute peritonitis. If the heart muscle does constantly receive new fibers from the sinoventricular bundle, it is reasonable to expect that there would have been more pronounced symptoms of cardiac insufficiency after the relatively long time that the ventricles had no connection with the sinus region. Furthermore, if there were a constant genetic progress of cells from the sinus to the ventricular myocardium, there would certainly have been marked changes in the myocardium, which were absent in this heart; and especially would there have been profound changes in the part of the conduction system below the lesion. Neither atrophic nor hypertrophic changes are mentioned occurring in the ventricles, nor is either change shown in the sketches accompanying the report. If there were no such changes resulting from the above-described lesion, such would be indirect evidence that the number of cardiac muscle fibers in the adult was the same as the number laid down at birth as stated by MacCallum ('98) and direct evidence against a replenishment of the myocardium from the sinoventricular bundle; for there was no atrophy in the part of the sinoventricular bundle below the lesion. Retzer himself was unable to find any histologic evidence of a degeneration of myocardium in many normal hearts examined.

Keith and Flack ('06) confirmed Tawara ('06) in his finding that this system neither enlarges with cardiac hypertrophy nor diminishes in size with atrophy. Beck and Stokes ('08) and Cohn and Lewis ('12), record the same findings as Keith and Miller as regards failure of the bundle to atrophy beyond a lesion. Erlanger and Blackman ('10) find that this system does not regenerate after its experimental destruction.

Through the kindness of Doctor Erlanger, we have had the opportunity of examining the sections of the ventricular septum of dog no. 8 described in his article to which we have just referred. There is no abnormality above the scar, no regeneration through or around the scar, and no atrophy below the scar. The atrio-ventricular bundle was almost but not quite completely crushed, as shown by the sections. It would seem that conditions here were favorable for at least a partial regeneration, but none occurred. This dog lived for three months and had complete heart-block up to the fifteenth day. From the fifteenth to the forty-second day the block was incomplete. Thereafter the block again became complete and remained so until the death of the dog. Erlanger and Blackman believe that this variable condition may be accounted for by the subsidence of inflammatory processes so that about the fifteenth day physiological connection was again partially established, but that on about the forty-second day the contraction of the scar tissue again broke the partial physiological continuity of the bundle, and again caused complete heart-block.

As regards the regeneration of the sinoventricular bundle, all the evidence shows that it does not regenerate after injury. This fact is also true of all cardiac musculature. Erlanger ('08) crushed the musculature of the atrial appendix in a carefully controlled and aseptic experiment. He found that there was no attempt at regeneration on the part of the myocardial fibers, but that nerve fibers readily regenerated across the contused area. Inasmuch as the atrioventricular bundle is known to be as much a part of the primitive heart as is the myocardium itself (Erlanger, '12) we would hardly expect any difference in this regard between them.

SUMMARY

1. The sinoventricular bundle is not embryonic muscle tissue. It would appear to be a specially differentiated tissue with the definite function of conduction of the impulse to heart beat.

2. There is no adequate histologic evidence in support of the suggestion that worn-out myocardium is replaced from the sinoventricular bundle.

3. Whether or not the sinoventricular bundle has retained the fundamental property of contractility is not to be determined by histological study; it must be determined by improved graphic physiological methods.

The writer is pleased to acknowledge his indebtedness to Dr. H. E. Jordan, University of Virginia, for his inspiration, aid, and invaluable criticism during the course of this work.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Drawing of a section of the sinus node taken parallel to the long axis of the right atrium. The nuclei are large, oval, or elongated, with a random distribution of fine chromatin granules and several nucleoli. Some cells are binucleated. The cytoplasm shows large perinuclear spaces. Striated myofibrils are distributed around the periphery of the cells. $\times 780$.

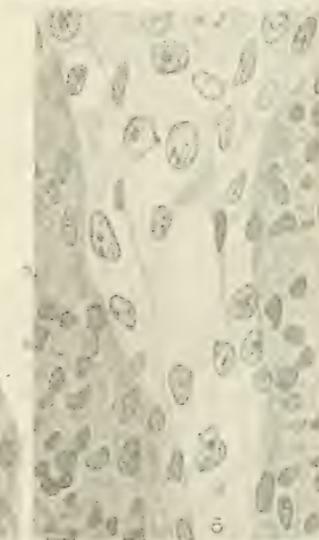
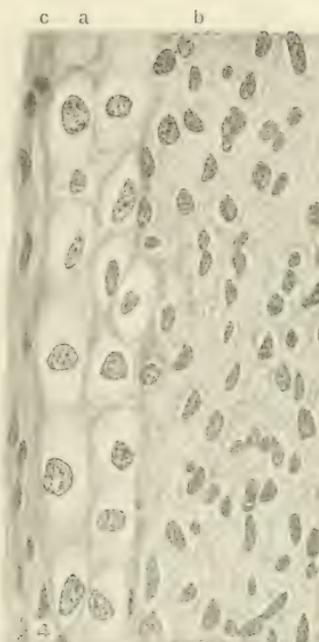
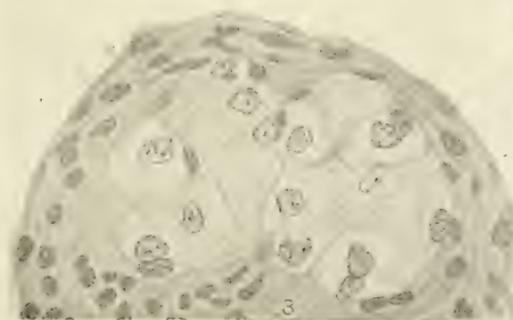
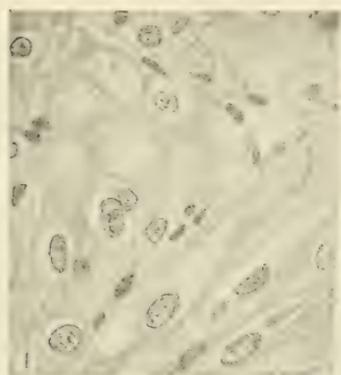
2 Drawing of a section of the atrioventricular node taken parallel to the long axis of the heart. Description same as in figure 1.

3 Drawing of a section of a false tendon cut at right angles to the long axis, showing: (a) The sinoventricular bundle tissue with the characteristic large oval or elongated nuclei. Many of the cells are binucleated, and the nuclei are closely adjacent to each other. The cytoplasm is pale and contains fine myofibrils in the cell peripheries. (b) Endocardium and connective-tissue sheath. $\times 780$.

4 Drawing of Purkinje fibers (a) in cross-section, showing relative differentiation as compared with adjacent ventricular myocardium (b). The nuclei are larger, paler, and more nearly oval. The perinuclear spaces are much larger than in the myocardium. The myofibrils are fewer, finer, and paler, and placed more peripherally than in the myocardial fibers. (c) Endocardium. $\times 780$.

5 Purkinje fibers in oblique section, showing gradual transition (a) into myocardial fibers (b). The nuclei gradually change in character from the typical Purkinje nuclei to the typical myocardial nuclei. The myofibrils gradually converge and pass insensibly into the myocardial fibers. $\times 780$.

All drawings are from hematoxylin and eosin preparations.



Resumen por el autor, R. Bennett Bean.

Observaciones sobre la enseñanza de la Anatomía.

I. Introducción. El profesor y el estudiante son más importantes que el método seguido, y la investigación es necesaria por parte de ambos. La anatomía comparada y la embriología deben requerirse como los fundamentos de la anatomía. El autor enumera algunos de los métodos seguidos por otros profesores, aconsejando la combinación de lo científico y lo práctico. Los estudiantes y las tradiciones difieren según las escuelas; los métodos empleados en una pudieran no ser útiles en otra. II. Métodos propios. (1) Cuadros murales de las partes fundamentales de los sistemas linfático, nervioso, simpático, alimenticio y arterial, con reconstrucción por los estudiantes son importantes para adquirir un conocimiento de conjunto más bien que un conocimiento fragmentario de la anatomía. (2) Los estudiantes aprenden los detalles y las relaciones al demostrar las partes disecadas a los instructores. (3) Un curso avanzado de anatomía aplicada con preguntas y demostraciones enseña al estudiante la manera de expresarse, y hace resaltar las partes importantes, especialmente la función y las aplicaciones para estudios futuros. (4) La Antropología se usa como un fundamento y un accesorio importante en la enseñanza de la anatomía. III. El autor ha enseñado tres tipos de la raza blanca, hiper y meso, braquiskele y macroskele, carnívoro y herbívoro en relación con los modos de variación, de carácter de la mente, y de la inmunidad y susceptibilidad para la enfermedad, durante los diez últimos años. Los tipos físicos han sido ya establecidos; los tipos psíquicos, fisiológicos y patológicos están aún en el umbral. La Antropología y la Psicología debían estar en la misma base que la Anatomía, fisiología, cirugía o cualquier otra parte un plan de estudios médicos bien repartido.

REMARKS ON TEACHING ANATOMY

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Many methods have been devised for the teaching of anatomy, and inasmuch as any method may succeed in suitable environment, the suggestion of other methods will give a wider range for selection. Choice of the best from the wide experience of the many, through trial and error, may ultimately result in a perfected system. Reticence is therefore not a commendable virtue, hence the present scattered remarks.

The teacher and the student, who are the two active elements in all teaching, do not depend so much upon method as upon the teacher and the student. Given fitness in both, and the task is half done. And the method that works well under certain conditions may not work well under others. Teachers and students vary.

The value of research is generally recognized as a prime requisite for the teacher of anatomy, and the attitude of the student should be that of a discoverer. A journey of exploration is to be conducted into the unknown. It were folly to attempt such a journey without guide or compass or without previous experience. Courses in comparative anatomy and embryology are as necessary to fit the student for the highest expression in anatomy as courses in Greek, Latin, and Anglo-Saxon to fit the student for the highest expression in English; yet the one is about as rare as the other.

The size and complexity of the human brain cannot be appreciated without having studied the brains of a series of animals; the homologies of the bones and muscles cannot be understood without a previous course in comparative anatomy; likewise other parts of the human body may be clarified, and what a difference in dissection if the student has already dissected a mammal!

Embryology is more important as a prerequisite to the study of anatomy than is comparative anatomy. The foldings of the brain; the separation of the hippocampus and the mammillary bodies from the frontal lobe; the development of the heart; the vestigial structures from the fetal circulation; the foldings of the peritoneum and of the alimentary canal, the fixation of the canal, its constrictions and dilatations, and the outgrowth of organs from it; the descent of the heart and diaphragm with the resultant position of the vagus and phrenic nerves; the non-descent of the testes; the relative length of the spinal cord and vertebral column resulting in the varying obliquity of the spinal nerves; the budding of the limbs and their growth as they drag along the muscles, nerves, and vessels to give them their ultimate course, relations, and distribution; to mention only a few of the multitudinous things that depend upon a knowledge of embryology will suffice to show that such knowledge is a prerequisite to the understanding of anatomy.

The great problem in teaching anatomy is how to combine the scientific and the practical in a subject of such vast detail and such multitudinous ramifications. There should be accuracy and thoroughness and at the same time the relation of anatomy should be taught as a foundation for anthropology, psychology, physiology, pathology, medicine, and surgery, each of which has equal value in a properly balanced medical course. It is inconceivable that in the time usually allotted to anatomy the student can make a careful dissection of all the structures of the body in their minutest details, learn these, and at the same time learn all the relations of anatomy to the six branches of the curriculum mentioned. The usual method is to do one thing or another, to teach anatomy as a science or to teach it as a trade, a tool for surgery. Some insist upon accuracy and thoroughness in dissection as the most important; others stress the relations to surgery and physical diagnosis; some teach 'physiological' anatomy; others 'philosophical' anatomy; some attempt digression into anthropology, some into pathology, others into psychology, still others make anatomy a basis for clinical medicine. Some advocate an entire revamping of the curriculum, and the

coordination in time of anatomy and other studies; whereas others would study by systems, so-called systematic anatomy, whereas still others would stress relations and topography. There is an element of truth, good sense, and right in almost all methods, yet no one can use all the good methods, nor can the good points of all methods be combined, but some good may be derived from many.

It seems necessary to insist on accuracy and thoroughness. The scientific side is important, therefore clean and careful dissecting is necessary, but the practical bearings of anatomy to subsequent study and practice cannot be overlooked. Probably the scientific and the practical are combined everywhere by different methods and with varying success.

The students and the traditions are different with the different schools, and that which gets results in one place might prove a failure in another. I have taught at places so absolutely different that the same methods throughout might have been disastrous. This may be illustrated by brief statements about each school where I have taught. The students at the Johns Hopkins must work independently. They are mature and have usually had courses in comparative anatomy and embryology. Their methods and habits vary, but the spirit of the place incites to drastic effort. Virginia students have the traditions of hard study and terrific examinations, through which have to be infused individual initiative and the perspective of research. The University of Michigan students have vigor and enterprise that need tone and direction; their traditions and environment lead naturally to research. The bigness of Tulane as the largest southern institution of medicine deepens responsibility and infuses ardor into the versatile students, but they need to become imbued with the value of research and the scientific spirit. The Filipino students have had twelve years or more of training in American schools, with two years in zoology, they come amply prepared, and, with their nimble wits and fingers, it is an easy matter to secure beautiful dissections, artistic reproductions by modeling, drawing, or painting, and to stir an interest in science. Unfortunately, flagging zeal or impaired health lead many aside.

It may not be amiss to give a few of the methods that have been used to apparent advantage.

The students, four to a subject, spend a short while on the thoracic and abdominal walls to familiarize themselves with the technique of dissecting and the recognition of structures. Then the abdomen and thorax are opened, the position of the organs is outlined in detail, the ramifications of the celomic cavities are explored, and the students make skeleton drawings of the basic parts of the alimentary and circulatory systems. This is supplemented later by the basic parts of the sympathetic and lymphatic systems, to which are finally added the peripheral parts as they are dissected. The body is turned over, and the basic parts of the bony, muscular and nervous systems are charted, to which may be added at least the peripheral nerves, thus securing charts of the great systems as a whole. Reconstruction of the principal pathways of the brain and cord as studied in cross-sections, and likewise of the organs from vertex to perineum are also required in their respective courses. Only by some such means as this does the student secure anything except a fragmentary or topographic idea of the body systems. The student learns the details and relations, and demonstrates them on the dissected part to the instructor.

It is especially desirable that the students reconstruct by chart the lymphatic system, because it is impossible to get any but a segmentary idea from dissecting the average cadaver, and the treatment is inadequate and incomplete in the text-books. The subject should be approached from two standpoints: the drainage of each organ or area, and the source of the lymph for any group of glands. Cancer of the liver secondary to cancer of the pancreas, pylorus, or mammary gland may be explained by the collateral circulation when certain groups of lymph glands are obstructed. The sources of infection for any group of glands should be known.

The charting of the spinal cord *in situ*, with the relation of the origin and exit of each nerve to the tips of the spinous processes and intervertebral discs, is illuminating to the student and the distribution in skin and muscle of the spinal as well as peripheral nerves is valuable in diagnosis.

Another method that has aroused enthusiasm among the students is the quiz-talk with demonstrations of prepared specimens, cross-sections, models, and charts. This method is especially valuable when a teacher has had considerable experience and the students have finished their dissection, and when given in connection with the course in topographic applied anatomy. An effort has been made to combine by adaptation the inimitable apt imagery of Osler with the illuminating magnetic method of Welch in drawing out students. A discussion of the number and value of valves in veins in relation to gravity and muscle action, the collateral circulation in lymphatics, bone growth, group muscle and nerve function, fascial sheaths, paralysis of nerves, anastomosis of arteries, blood and nerve supply of bone and joint, the mechanism of joints and of the pelvis in growth and sex, the lower extremity in stability and progression, the upper in delicate manipulation, to give but a few illustrations, serve to stimulate search beyond the text-book. Function is emphasized and the relations to medicine and surgery are stressed.

Beyond all else the broad fundamentals of anatomy are to be found in physical anthropology, the type and the race. Until the teaching of anatomy has its foundation in the types of man, it cannot be presented truly as it exists. There are many means of differentiating types, but the ear form is one of the most uniquely distinctive differentiators. I have been able to prove by the ear form alone differences of three meters in the length of the small intestine, of 500 grams in the weight of the liver of smaller amounts in the size of the brain, cerebellum, heart, kidneys and spleen; and difference in the shape and altitude of the organs in the abdomen; and in addition, if different human types represent different forms of intellect and differences in susceptibility and immunity to disease, then anthropology becomes the handmaiden of anatomy, an essential adjunct in its teaching.

Adult human types probably represent the end-products of chemical reactions that have been continuously at work throughout the life of the individual, or at least a large part of the life.

It is only fair to assume that the net result of this activity will be easier to perceive than the chemical reaction at any particular moment. It may be fruitless to attempt to determine or differentiate chemical types, but physical types have been established.

Anthropology has already established types of men, and at no distant date types of minds will also be established. These will play an ever-increasing part in medicine as their value is realized. Medicine has become one-sided through the study of the rôle of bacteria and kindred disease producers, to the neglect of the physical and psychical types and conditions. It is unnecessary to entail a discussion of the varying share of the incitor and host in the production of disease, or upon the immunity or susceptibility of the individual due to type or mental state, and it would be beside the mark. Once recognize the equal importance of the germ and of the man, the full value of the physical and psychical type and state, then follows as night the day the introduction of courses in anthropology and psychology on a par with pathology, physiology, or anatomy.

Dr. Goldthwaite, in the Shattuck lecture for 1915, presents the types of man as a basis for diagnosis and treatment, as do Percy Brown and Bryant. There is also an editorial in the number of the *Journal* which has the Shattuck lecture, wherein, with prophetic vision, the editor states that some medical school will give a course in anatomy based upon human types, others will follow until the custom becomes universal. Until anatomy is taught with the human types as a basis, it will not be taught as it exists. The structures of the body are found to vary about modes, each of which is normal and none of which is the average.

The writer has taught anatomy from the standpoint of types of men during the past ten years, both at Tulane and Virginia, during which time about 1,000 students have passed through this instruction. Other schools have in the meantime shown an interest in anthropology, as at Western Reserve and Washington University, and we may confidently look forward to the time when human types will be understood by the practitioner.

Such a piece of work as that published in *L'Anthropologie* by Dr. L. and Madame H. Hirschfeld may interest physiologists, pathologists, and internists. Serum tests were made during the Great War on about 500 soldiers in each of many national groups of Europe, of Asia, and of Africa, and differences that amounted to more than 50 per cent were found. The tests were so acute and positive that individual heredity could be determined, the parentage of any child verified.

Manouvrier and Godin have established types by skeletal form and through anthropometry on growing children, living adults, and the cadaver. Inspection aided by anthropometry will readily differentiate the types.

Chaillon divides the types into four: digestive, respiratory, muscular, and cerebral, from the physiological and clinical standpoint.

Mills has two types of visceral form, hypersthenic and asthenic, as determined through the x-ray by position, tonus, and motility. Bryant, following Treves and others, has the carnivorous and herbivorous types, as determined by the functions of the alimentary canal and by diet.

The phthisical and plethoric habitus are familiar to many physicians.

The temperaments, as depicted by Albrecht Dürer in the forms of four apostles and as taught at the school of Salernum, based upon the four elements and upon the four humors of Hippocrates and known as the melancholic, choleric, phlegmatic, and sanguine, are in disrepute among modern scientific physicians, but there is probably an element of truth in all. The evidence needs to be sifted, the physiological, psychological, and pathological characters of the physical types assorted and verified.

It still remains to be seen whether the hyperphylomorph, the macroskele, the cerebral, the asthenic, the narrow back, the habitus phthisicus, and the carnivorous are identical; likewise whether the mesophylomorph, the brachyskele, the digestive, the hypersthenic, the broad back, the plethoric, and the herbivorous are identical. The anthropologist is fast settling the basic

features of the physical type: it is high time for the psychologist, the physiologist, and the pathologist to get busy, to associate or dissociate the mental, functional, pathological, and physical types.

We cannot hope that anthropology and psychology will come into their own immediately as separate departments in the medical curriculum, which is already overcrowded, but short excursions into anthropology by the anatomist must suffice to impress upon the student the verity of the types and their relations to the practice of medicine. The students themselves serve as excellent models for the demonstration of the types.

We, in America, do not yet appreciate the scope of anthropology; only a few universities in this country provide for courses, but when we recognize its value then we may approach the variety and extent of the courses given at the *École d'Anthropologie de Paris* with its fifteen or more departments, each with its staff and laboratories.

The course in anthropology or psychology should have its place in the medical curriculum as a separate department with its corps of instructors, and the courses should be correlated with anatomy, physiology, pathology, and medicine.

Let us hope that the study of types of man will be pursued diligently in many directions, and that a place and time will be found in the medical curriculum, as the need and demand become imperative through the diffusion of knowledge.

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Resumen por el autor, E. V. Cowdry.

Una comparación de las antiguas láminas anatómicas chinas con la "Fünfbilderserie" de Sudhoff.

Sudhoff y sus discípulos han descubierto una serie de cinco diagramas tradicionales de los sistemas muscular, esquelético, nervioso, venoso y arterial en las bibliotecas europeas y en manuscritos persas en las siguientes localidades; 1) Claustro de Profening, Baviera, 1158 D. J.; 2) Claustro de Seheyern, Baviera, 1250 D. J.; 3) Biblioteca de la Universidad de Basilea, unos 1250 años D. J.; 4) Biblioteca Bodleiana, Oxford, 1292 D. J.; 5) Misma biblioteca, unos 1340 años D. J.; 6) Oficina de la India, Londres, antes de 1400 D. J.; 7) Raudnitz, Bohemia, siglo XIV. Aunque no idénticos, se corresponden en tal grado que Sudhoff está justificado al deducir que todos ellos provienen del mismo sitio, probablemente de Alejandria. Los esquemas chinos coleccionados por el emperador K'ang Hsi (1661-1722 D. J.) y atribuidos por él a la dinastía de Han (206 A. J. a 220 D. J.) se asemejan a las "Cinco series de figuras" de un modo general en lo referente a la forma y proporciones, y posición forzada del cuerpo. Son mas primitivas y antiguas. Si existe alguna relación entre ellas hay razones para suponer que las figuras chinas son el original, las cuales a causa de su antigüedad carecen de detalles.

A COMPARISON OF ANCIENT CHINESE ANATOMICAL CHARTS WITH THE 'FÜNFBIldERSERIE' OF SUDHOFF

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SIX PLATES (TWENTY-FOUR FIGURES)

After reading the MS. of Dr. Hsieh's "Review of Ancient Chinese Anatomy," Col. Fielding H. Garrison was kind enough to suggest to me that some of the diagrams illustrated may perhaps be related to the 'Five Picture Series' of Sudhoff. Generous help from Professor Hu Suh of the Government University from our instructor in Chinese, Mr. Ma Kiam, and from Dr. E. T. Hsieh, has made it possible for me to examine many Chinese drawings and to establish dates accurately.

I am also indebted to the librarian of the National Library (1) for the unusual privilege of being permitted to enter the library itself and to examine the originals personally. Through the kindness of Mr. R. F. Johnston, tutor to the Emperor (2), a card of introduction was obtained to Mr. Ch'i Ling (3), a minister of the household who with great courtesy introduced me to Mr. Fu Ch'i (4) who, in turn, allowed me to consult the private library of the imperial family in the Forbidden City. Mr. Ch'i also arranged visits to the office of the imperial physicians (5), which is maintained by grants from the emperor who receives yearly \$4,000,000 from the Republic as an honorarium in consideration of his partial abdication. Here 110 physicians are registered, none of whom have any foreign training. Two of them visit the court twice a week and make prescriptions when necessary. Their official text-book, the 'Golden Mirror' (6), is the most interesting book I have found. Attached to the office of the imperial physicians is a small temple where an image of Huangti, the father of Chinese medicine, is worshipped during the spring and autumn festivals.

Sudhoff's series of five traditional diagrams representing the muscular, skeletal, nervous, venous, and arterial systems, was discovered by him in different European libraries and in Persian MS. as follows:

The Cloister of Prüfening near Ratisbon, Bavaria, (Munich, 13002), 1158 A.D.

The Cloister of Scheyern, Bavaria (Munich, 17403), 1250 A.D.

The Basel University Library (Provençal MS., D, II, ii), about 1250 A.D.

The Bodleian Library, Oxford (Ashmolean, 339), 1292 A.D.

The Bodleian Library, Oxford (Codex 19) about 1340 A.D.

India Office, London (Persian MS. 2296), before 1400 A.D.

The library of Prince von Lobkowitz, Raudnitz, Bohemia, (VI, Fe, 29) 14th century.

Specimens of the Persian and Provençal series are reproduced in plate 1.

Although the drawings in the different series are not identical, they correspond so closely that Mortimer Frank is justified in saying (p. 53) that "It may be asserted with almost historic perspicuity, that these figures with their text must have been based upon a short illustrated Alexandrian text-book of anatomy which was written in Greek and provided with schematic drawings done probably after representations then extant." Sudhoff feels that they were originally drawn in Alexandria.¹ Whatever their source, they influenced the teaching of anatomy in Europe until human dissection came to the rescue, as it is now doing in China.

In more recent European drawings the squatting posture, characteristic of these diagrams, is lost, they have become, as Mortimer Frank remarks (p. 58), free of the "constrained posture of centuries and present an easy pose." The same author inserts the following footnote:

"Dr. Berthold Laufer, of the Field Museum at Chicago, discovered during his travels four Tibetan anatomic plates, which

¹ Through the courtesy of Colonel Garrison, I was at first able to examine Sudhoff's original papers in the Surgeon-General's Library, but since my return to China I have had to rely entirely upon Mortimer Frank's review of Sudhoff's work.

evidently belong together and are still in use by students in Tibet today. They recall, in their squatting posture, so many of the details of the Prüfening and Persian figures that one is forced to believe that the well-known post-Alexandrian anatomic figures were brought to India also and that the Indian and Chinese doctrines went through a peculiar process of amalgamation whose later results still confront us in the Tibetan plates."

Unfortunately, I have not had an opportunity to examine these plates, but Surgeon-General Chuan, who has spent two years in Lassa, tells me that he has seen a large number of Tibetan anatomical plates and believes them to have been introduced from China. Moreover, Heinrich Laufer (p. 11) calls attention to the fact that Tibetan historical classics, written in 630 A.D., record the derivation of Tibetan medicine from China.

The Chinese diagrams which I have to report bear a certain resemblance to the 'Five Picture Series' of Sudhoff, as may be seen by reference to plate 2 in which figures 5 and 6 from the Raudnitz series are compared with figures 7 and 8 from the 'Golden Mirror' which is a compilation by the Emperor K'ang Hsi (1661-1722 A.D.) of earlier works on anatomy attributed by him to the Han Dynasty (206 B.C. to 220 A.D.). The two diagrams illustrated are exact copies of drawings which I found in a MS. entitled 'Leiching' (7) written by Chang Chieh Pin (8) about 1500 A.D. in the library of the imperial family.

1. In both European and Chinese series the entire body is taken as the unit and is represented by a simple outline drawing with very little attempt at perspective.

2. The peculiar squatting posture is evident in both, though it is perhaps more marked in the European figures.

3. With characteristic Chinese sense of propriety, the external genitalia are omitted in both series.

4. In the Raudnitz drawings the feet are abducted (turned laterally) almost to the point of dislocation, as in Chinese classical drawings. This abduction is illustrated to a less extent in K'ang Hsi's figures.

5. The general proportions of the body are alike. The trunk is longer than the legs, as in infants. Relatively short legs

(femora), though of course not exaggerated to this extent, are a feature of some Orientals, particularly of the Japanese. As far as I can ascertain, with our limited library facilities, ancient Egyptian illustrations depict figures which, far from being stubby in appearance, have relatively long legs.

From a large number of other drawings I have selected figures 9 and 10 for further comparison with the Raudnitz series. They are taken from a book on acupuncture and moxa (9) by Yang Chi Chow (10) which was written between 1573 and 1627 A.D. and consists of a collection and critical digest of older works. It antedates the 'Golden Mirror,' but has not received the same official recognition. It will be noted that the feet are completely abducted and thus correspond more closely with those in the Raudnitz series. The arrangement of the traveling vessels varies, as compared with the 'Golden Mirror' figures, but the general proportions of the body are maintained with typical conservatism. Compare also figures 11 and 12 from a book by Hsia Ting (11) on 'Yin Ke Tih Ching' (12) written during the reign of the Emperor Tao Kuang (1821-1850 A. D.), and figures 13 to 16 from the 'Hsi Yuan Lu Pien Ching.'

Even if we suppose a genetic connection between certain Chinese anatomical diagrams and those of Sudhoff, it would perhaps be too much to expect a very close resemblance, owing to the great difference in time and space. Nevertheless, the correspondence between K'ang Hsi's illustrations (figs. 7 and 8) and the Raudnitz series (figs. 5 and 6) is almost as close as that between the Persian drawings (figs. 1 and 2) and the Provençal series (figs. 3 and 4). It is only when we come to compare the details of the arrangement of the vessels and nerves that the similarity breaks down. Sudhoff is able to support his contention that his different series spring from a common source by the discovery that a similar Latin text often accompanies them, but the idea expressed in the Chinese diagrams seems to be radically different from the Western conception.

Each of Sudhoff's series consists of at least five drawings representing the muscular, skeletal, nervous, venous, and arterial systems, for which few counterparts can be found in Chinese

literature. Chinese anatomists did not distinguish between arteries and veins, and very seldom specifically referred to nerves. The skeletal system is occasionally illustrated, as in figures 13 and 14, which are reproduced from a book by Chu Chang Yung called 'Hsi Yuan Lu Pien Ching' (13) said to be an exact copy of a Sung Dynasty (960-1127 A.D.) original. In figure 14, showing the skeleton from behind, the author, with the usual disregard of detail, is content to represent the areas in a schematic way, not troubling himself to illustrate the individual bones. This is particularly noticeable in the hands. I have been unable to find any diagrams of the muscular system. Perhaps this is explained by the circumstance that the Chinese as a race see nothing beautiful in the play of muscles in the body in motion. Their artists, from very early times, have devoted all their attention to color values and to the representation of draped figures. How different from Greece where, as Garrison and Streeter remark (p. 375), nudity was the 'festal costume'! The Greeks acquired their knowledge of musculature "not from dissection, but from empirical observation of athletes in action during games and military exercises." European artists took a very active interest in anatomy, as may be seen from the following statement of Vesalius, which I also quote from Garrison and Streeter (p. 394):

"As for those painters and sculptors who flocked around me at my dissections, I never allowed myself to get worked up about them to the point of feeling that I was less favored than these men, for all their superior airs."

What the Chinese lose in the variety of the systems illustrated they make up for by the extent to which they are still saturated with the doctrine of circulating 'humors.' In this respect they resemble other primitive peoples. Typical diagrams of this kind illustrating the course of the male and female principles have already been mentioned (figs. 7, 8, 9, and 10).

With regard to the squatting posture, one of my students, Mr. Li Ke Ming, has made the interesting suggestion that the use of the diagrams for so many hundreds of years as acupuncture charts may be the determining factor, since the needles are

usually inserted while the individuals are sitting down. It will be noted that the position of the patient awaiting treatment in figure 17 is almost identical with the position of the body in the Persian diagrams (figs. 1 and 2). The stretching out of the arm in figure 18 and the frog-like position of the legs in figure 19 also reveal important acupuncture spots and call to mind figures 3 and 4 from the Provençal series. On the other hand, the body is often shown in other positions in order to illustrate conveniently other acupuncture spots, so that this similarity in position may after all be a mere coincidence. It should also be remembered that Chinese artists are accustomed, in their classical portraits, to represent the knees widely spread apart and the toes turned outward.

Other less plausible explanations suggest themselves. Perhaps the diagrams were used long ago as charms to discourage innumerable evil spirits. A drawing reproduced from de Pourvoirville by de Zwaan is interesting. It represents a skeleton in the same squatting attitude which gives it a ferocious and terrifying appearance (fig. 20). The posture is more constrained than that of Persian drawings. It is Japanese (Stratz, p. 126) and relatively modern.

It is difficult to determine the actual age of the Chinese diagrams. They are usually attributed to the mythical Yellow Emperor, Huangti, who, according to tradition, ruled in China about 2696 B.C. Neuburger, however, in common with modern Chinese historians, is sceptical regarding this assumption. Unfortunately, we know very little of Huangti. E. T. Williams tells us that, according to de Laeouperie, he was the leader of a band of immigrants entering China from the west, and remarks that "while several traditions associate the name of Huangti with the Province of Kansu, he was probably born beyond the western boundary of China." According to Li Ung Bing (22), 'real authentic history' begins with the Chou Dynasty (1122-255 B.C.). Writing seems to have been invented considerably earlier, though Zelia Nuttall and Elliot Smith (p. 17) question whether the art of writing was known in China before the sixth century B.C. The Chinese classics tell us that writing became

so complicated that an attempt at standardization was made in B.C. 868 when 3300 characters were officially recognized (Ross, pp. 115, 117). They remain to us as 'seal characters' on bronzes attributed to the Chou Dynasty. In the opinion of sinologues, exact correspondence in dates extends back to August 29, 776 B.C., when the same eclipse was observed and recorded both in Europe and China (Hirth, p. 174). Since earlier dates may easily be determined by computation, it is fair to assume, with Hirth, that "we should date the commencement of the historic period, as far as the main facts are concerned, many generations before Yu-wang (781-771 B.C.), while making allowance for doubts in the chronology owing to the two-fold tradition."

I have been unable to obtain clear-cut evidence of the existence of any anatomical diagrams before the Han Dynasty, 206 B.C. to 220 A.D. The diagrams which I have illustrated are for the most part fairly recent prints of older originals. They can be traced back as follows:

Figures 7 and 8, Han Dynasty, 206 B.C.-220 A.D.

Figures 9 and 10, 1573-1627 A.D.

Figures 11 and 12, 1821-1850 A.D.

Figures 13 to 16, Sung Dynasty, 960-1127 A.D.

Figures 17 to 19, Sung Dynasty, 960-1127 A.D.

Figures 21 to 24, Sui Dynasty, 581-618 A.D.

It is not improbable, however, that they existed still earlier in the form of traditional sketches, emanating perhaps from central Asia, where Pumpelly has brought to light interesting remains, dating back, according to Professor Ulrich Duerst, of Berne, to about 8000 years B.C. A Western origin of the Chinese people is claimed by Pére Richard, de Lacouperie, and others, and vehemently denied by Giles, Hirth, and Ross.

Since, however, Mortimer Frank has suggested that Tibetan anatomical plates, which closely resemble these Chinese diagrams, are derived from Sudhoff's hypothetical Alexandrian originals, we have to consider carefully the possibility of Egyptian derivation.

According to Elliot Smith, there is "sufficient information to justify the conclusion that many of the fundamental conceptions of Indian, Chinese, and Japanese civilization were planted

in their respective countries by the great cultural wave which set out from the African coast not long before the sixth century B.C."

In this connection it cannot be denied that the anatomical diagrams reproduced in plate 6 exhibit a distinctively Egyptian appearance. The profile view with the prominent nose is remarkable since it is so different from what we ordinarily meet with among Chinese. The nose in figure 21 seems to be almost semitic. Figures 22 and 23 illustrate perhaps the Lange 'law of frontality,' referred to by Garrison and Streeter (p. 372), for they gaze 'directly and rigidly forward,' as in Egyptian statuary. They are also disposed as in a bas-relief. Mr. Ma Kiam and Mr. Ch'i Ling inform me that the style of dress in figure 22 is certainly not Chinese. The skirt of figure 23 is suggestive of a sketch on one of the pillars of the Hypogeum of Seti I, as reproduced by Maspero (p. 148). The coiffure is apparently of about the Han Dynasty (Ma Kiam).

These four drawings are from a book by Hsi Fang Tzu (14) called 'Ming Tang Chih Ching' (15). This is a print of an original published in the Sung Dynasty (960-1127 A.D.) which I have examined in the National Library and found to be similar, at least as far as the drawings are concerned. They are often mentioned in Chinese history. 'Ming Tang' is, according to Professor Hu Suh, a generic term, of unknown origin, applied to practically all anatomical diagrams. The policy of the Emperor T'ai Tsung of Tang (627-649 A.D.) is said to have been influenced by an examination of the 'Ming Tang' diagrams. They are also mentioned in the bibliographical sections of the Histories of Sin (16) and of Tang (17). He is of the opinion that they have been employed as anatomical diagrams, illustrating the points of application of moxa, from the beginning and that they are not simply old originals recently used for this special purpose. The notation is unusual, because the reference lines do not indicate areas limited by small circles as in the other drawings which I have reproduced.

They may have been introduced into China by sea in the manner suggested by Elliot Smith. They may also have come overland, for, as Fenollosa remarks, Chinese military domination was

felt as far west as the Persian Gulf during the Han Dynasty, and rock carvings in Shantung caves made at this time display in his opinion, primitive Egyptian designs. It is not difficult to find other suggestions of Egyptian influence (Barbezieux-De Zwaan, p. 137).

Giles refers to some old stone sculptures collected for a mausoleum by the Wu family in 147 A.D., which are still the subject of considerable controversy. Douglas feels that they show unmistakable Egyptian influence, which, however, Paleologue and Chavannes deny (Giles, p. 12). Chavannes invokes the theory of 'convergence' in the following words:

"En fait, on découvra des rapports entre les premiers essais artistiques de tous les peuples parce que partout les mêmes causes produisent les mêmes effets; mais il faut se rappeler que, par un corollaire de ce même principe, ressemblance n'implique pas filiation."

Since the drawings are not, however, of the squatting type, they only influence our general problem by indicating the possibility of the Egyptian derivation of the squatting figures, for which I know of no other evidence.

It is possible that transference took place in the reverse direction, from China to Egypt, either overland or by sea. The Phœnician mariners could hardly remain uninfluenced by their contact with the Orient. A few centuries later Arab traders (de Goeje) carried back, among other things, Chinese bottles containing classical quotations which may be identified (S. W. Williams) and from which I select the following:²

Wang Wai, 702-745 A.D.

Wei Ying-wuh, 831-837 A.D.

Su Tung-po, 1068-1085 A.D.

These and other 'cultural waves' may have influenced life and thought along the Nile at about the time that Sudhoff considers the originals of his 'Five Picture Series' to have been drawn. It is even possible that rough diagrams representing the body in

² Obviously, they were brought to Egypt after the dates mentioned and probably before the end of the Sung Dynasty (1127 A.D.) when the Arab trade with Canton and Hangchow commenced to decline.

the squatting position were introduced from China about this time, or earlier, which perhaps served as a basis on which the Alexandrians grafted their more accurate knowledge. According to Mr. Ch'i Ling, there is a tradition, hazy it is true and incapable of verification, but none the less insistent, that in the old days Chinese anatomical diagrams were sent to the peoples of the West. Certainly, the Chinese drawings are older and more primitive than the European and Persian series thus far discovered. If any relation exists between them there is every reason to suppose that the Chinese are the originals which by reason of their age are wanting in detail.

- | | | | |
|----|-------|----|---------|
| 1 | 京師圖書館 | 12 | 幼科鉄鏡 |
| 2 | 宣統 | 13 | 洗冤錄 辨正 |
| 3 | 耆齡 | 14 | 西方子 |
| 4 | 福啟 | 15 | 西方子明堂灸經 |
| 5 | 太醫院 | 16 | 隋 |
| 6 | 醫宗金鑑 | 17 | 唐 |
| 7 | 類經 | 18 | 瞿中溶 |
| 8 | 張介賓 | 19 | 宋淳祐 珠 |
| 9 | 鍼灸大成 | 20 | 瘡瘍經驗 |
| 10 | 楊繼洲 | 21 | 竇漢卿 |
| 11 | 夏鼎 | 22 | 李文彬 |

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PLATE 1

EXPLANATION OF FIGURES

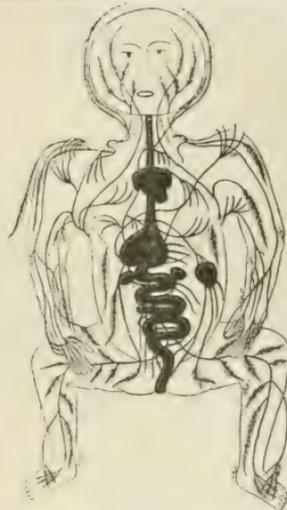
Examples of the 'Five Picture Series' of Sudhoff

- 1 Arterial system, from Persian MS., no. 2296, in India Office, London. (From Frank's translation of Choulant's history and bibliography of anatomic illustration. Originally from Sudhoff's *Geschichte der Anatomie im Mittelalter*, Leipzig, 1909, plate V.)
- 2 Venous system, from Persian MS. no. 2296, in the India Office, London. (Reproduced from same source as fig. 1.)
- 3 Arterial system, from a late thirteenth century Provençal MS. (D II ii) in the Basel University Library. (Reproduced from same source as fig. 1.)
- 4 Male generative system from a Provençal MS. in the Basel University Library (D II ii). (Reproduced from same source as fig. 1.)



ARTERIAL SYSTEM, FROM PERSIAN MS. NO. 2266, IN THE
INDIA OFFICE, LONDON

1



VENOUS SYSTEM, FROM PERSIAN MS. NO. 2266, IN THE
INDIA OFFICE, LONDON

2



ARTERIAL SYSTEM, FROM A LATE THIRTEENTH-CENTURY PROVINCIAL MS (D. II. 41)
IN THE BASIL UNIVERSITY LIBRARY

3



MALE GENERATIVE SYSTEM, FROM A LATE THIRTEENTH PROVINCIAL MS (D. II. 41)
IN THE BASIL UNIVERSITY LIBRARY

4

PLATE 2

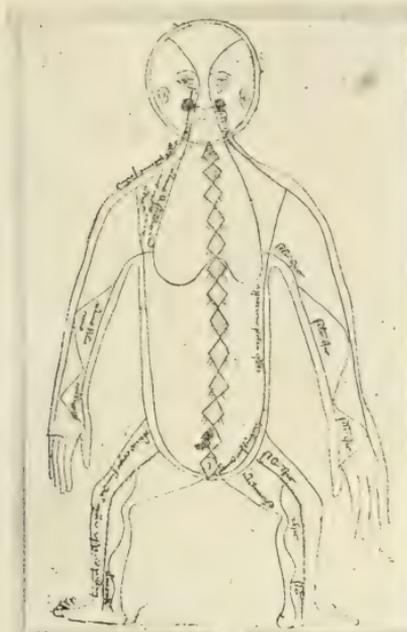
EXPLANATION OF FIGURES

Comparison of the 'Five Picture Series' (figs. 5 and 6) with drawings edited by the Emperor K'ang Hsi (figs. 7 and 8)

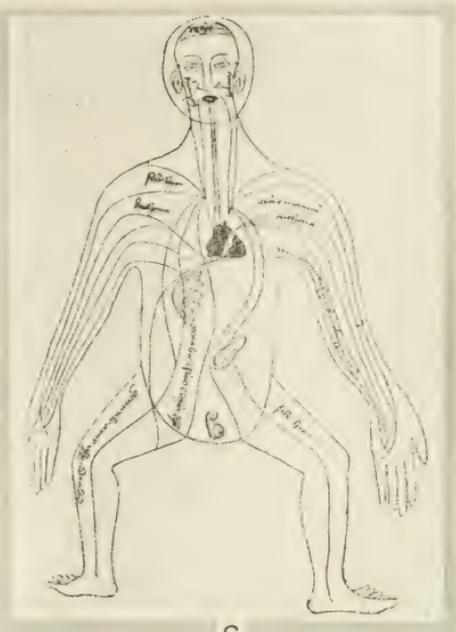
5 Nervous system, from a fourteenth-century MS., in the library of Prince von Lobkowitz (Raudnitz, Bohemia). Reproduced from Sudhoff: *Arch. f. Gesch. d. Med.*, Leipzig, 1909-10, Bd. 3, Pl. XI.

6 Arterial system, from a fourteenth-century MS. also in Prince von Lobkowitz's library, from Sudhoff: *Ibid.*, Pl. IX.

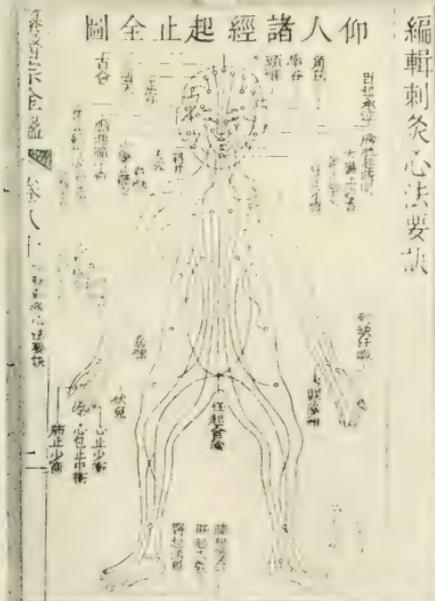
7 and 8 Diagrams of the course of the traveling vessels, after Hsieh. Compiled by the Emperor K'ang Hsi (6) (1661-1722 A.D.) from originals attributed by him to the Han Dynasty (206 B.C. to 220 A.D.).



5



6



7



8

PLATE 3

EXPLANATION OF FIGURES

Examples of old Chinese diagrams

9 and 10 Diagrams illustrating the course of the traveling vessels from a book on acupuncture and moxa (9) by Yang Chi Chow (10) written between 1573 and 1627 A.D. The drawings are certainly copies of older originals.

11 and 12 Acupuncture charts from a book by Hsia Ting (11) entitled 'Yiu K'e Tih Ching' (12) written during the reign of the Emperor Tao Kuang (1821-1850 A.D.), probably derived from older works.

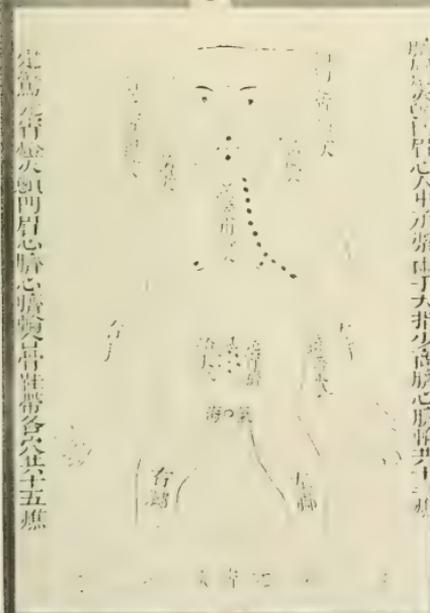
E. V. COWDRY



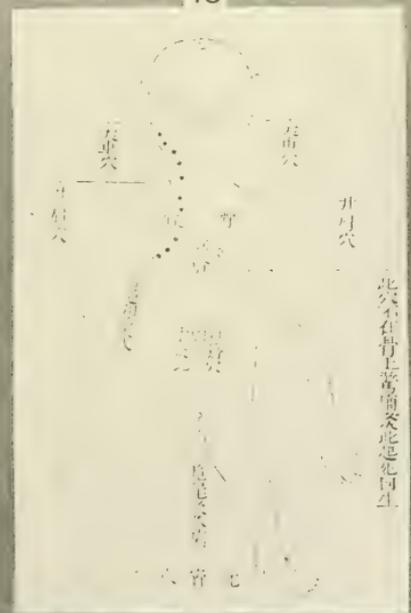
9



10



11



12

PLATE 4

EXPLANATION OF FIGURES

Further examples of old Chinese diagrams

13 Skeletal system from a book by Chu Chung Yung (18) entitled 'Hsi Yuan Lu Pien Ching' on legal medicine (13), said to be a new and revised edition of a Yuen edition (19) of the 'Hsi Yuan Lu,' originally published in the Sung Dynasty (960-1127 A.D.).

14 Skeletal system, same source. Having already illustrated the anterior view, the author does not feel it necessary to fill in the individual bones as seen from behind, so he merely represents the areas concerned in a schematic way.

15 Acupuncture spots, anterior view, same source.

16 Acupuncture spots, posterior views, same source. The black circles are shown in red in the original and are supposed to indicate places where mortal wounds may be inflicted.

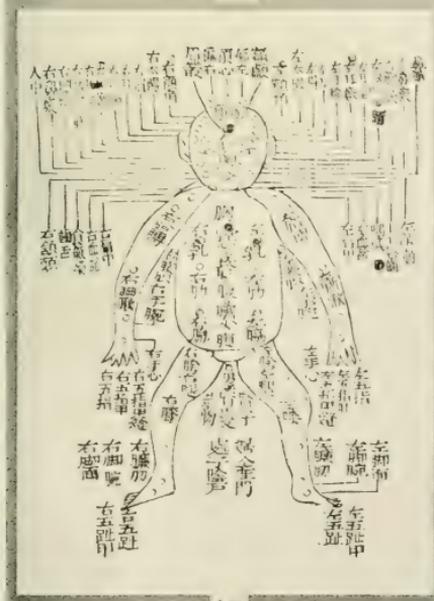


PLATE 5

EXPLANATION OF FIGURES

Drawings illustrating the possible origin of the squatting position

17 Patient awaiting treatment. From a book called 'Chuang Yang Ching Yen' (20), by Premier Do (21), of Sung, published during the reign of K'ang Hsi (1661-1722 A.D.), said to be a copy of a Ming edition of the Sung book. Note the similarity between this position and that shown in the Persian sketches (figs. 1 and 2).

18 Acupuncture diagram from a book by Chang Jea Ping (8), also a copy of a Sung original. Note how the position of the arm and hand corresponds with figure 6 of the Raudnitz series.

19 Acupuncture diagram from the same source in which the position of the legs is suggestive of the Provençal series (figs. 3 and 4).

20 Relatively modern drawing of a Japanese skeleton in extreme squatting attitude from de Zwaan.



以住肌致致生此直赤腫走注不定用溫水

真六寸骨

17



天泉
印門
關從
大陵
中衝

18



足厥陰肝經
左右共一十六穴

期門
陰廉
五里
中衝
大陵
中衝

19



20

PLATE 6

EXPLANATION OF FIGURES

Drawings suggestive of Egyptian derivation

21 to 24 Sketches from a book by Hsi Fang Tzu (14) called 'Ming Tang Chih Ching' (15), reprinted from an original published in the Sung Dynasty (960-1127 A.D.). They are frequently mentioned in Chinese history. 'Ming Tang' is, according to Professor Hu Suh, a generic term of unknown origin applied to anatomical diagrams in general. The policy of the Emperor T'ai Tsung (627-649 A.D.) is said to have been influenced by an examination of 'Ming Tang' diagrams. They are also mentioned in the bibliographical sections of the Histories of Sin (16) and of Tang (17) and probably date back to the Sui Dynasty, 581-618 A.D.



21



22



23



24

Resumen por el autor, William Martin Smallwood.

Notas sobre una ternera con dos cabezas.

El presente trabajo es una descripción de las modificaciones estructurales a consecuencia de la duplicación de la cabeza. Una fotografía obtenida mediante los rayos X revela la notable semejanza de los dos cráneos, no solo en el carácter general de los huesos, sino en sus pequeños detalles estructurales. Los dos cerebros no son del mismo tamaño, difiriendo también en la distribución de las circunvoluciones y en la médula; están unidos entre sí en la región del puente y la médula oblonga. En las dos lenguas existe el mismo número de papilas circunvaladas en vez del número doble, como sería de esperar.

Translation by José F. Nonidez
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NOTES ON A TWO-HEADED CALF¹

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TWO TEXT FIGURES

Wilder and Johnston have each, especially the former, described 'double monsters,' yet a brief note on another is desirable, as a more detailed record is possible in this case than in others of a similar kind.

On the 25th of December, 1920 a registered Holstein, Kate Koningen De Kol, gave birth to a two-headed heifer, one week short of term. The cow is owned by W. H. Kinney, Elbridge, New York.

The calf was large and well formed except for the double head. It lived seventeen hours and was able to move the jaws independently. When given milk through one mouth, the milk escaped from the other. This was due to the condition of the esophagus which was closed. It was impossible to force a small probe from the opening of the esophagus in the neck into the mouth, the closure being opposite the larynx. The mounted specimen is in the museum of the College of Agriculture, Syracuse University.

A dissection of the specimen revealed that the organs of the body were normal and that the blood vessels of the neck showed no marked variation until the head was reached.

Johnston ('01) concludes his study of thirteen two-headed snakes with the statement that the point of bifurcation of the vertebrae is more posterior than would be supposed from external examination. The skulls frequently appear united externally when in reality they are not. Reviewing the literature, Johnston also states that the evidence certainly shows that there was a doubling of internal organs caudad to the vertebral bifurcation.

¹ Contributions from the Zoological Department in the Liberal Arts College, Syracuse University, C. W. Hargitt, Director.

This is not the case in either the vertebrae or other organs in the specimen under discussion. There is a single larynx with two folds of the soft palate and a single epiglottis. There is a single hyoid chain of cartilages as in a normal animal. The cartilages of the large larynx are also normal and there is no indication of morphological variation in this organ. Although the bleating of the calf escaped from either mouth, the sound came from a single organ.

The four incisors on each of the four mandibles are present, well developed and symmetrically distributed. These simple temporary teeth have their shovel-shaped crown narrower than normal. This is more pronounced in the 'corner incisors,' each of which is nearly conical, suggesting their possible canine origin.

Four deciduous premolars are clearly defined and correspond in proportionate size to these same teeth in a normal calf. A section of a normal mandible permits one to note the nature of the roots and compare them with the x-ray photograph (fig. 1). The clearly defined mandibular canal is similar in each mandible, with the roots of the incisors and premolars showing the same orderly arrangement as in a normal jaw.

In the dicephalous lamb described by Miss Bishop the mandibles on the inner side of the two snouts are only half as long as the outer ones from symphysis menti to rami. Nor do these mandibles articulate with the temporal bone.

In this specimen there is very little difference in the length of the four mandibles. The two inner are more slender, but still articulate with the temporal bone, while the outer ones are distinctly curved and slightly longer.

Three deciduous premolars are present on each upper jaw and alike in minute details. The space where the first permanent molar will form shows as a light area immediately posterior to the third deciduous premolar. We were at first inclined to believe that there were two teeth lacking, but a study of several normal calf skulls shows a similar variation.

The two skulls are identical in general shape and size. They are attached by the fusion of the temporal bones, which are much thickened. In fact, the thickened roof of the skull was very apparent when removing the brain.

The x-ray picture reveals that the several sinuses are clearly defined and of equal extent, even to details. As nearly as I could judge, the two heads were joined to the vertebral column on the same axis. There is nothing to indicate from a study of the skulls that one of the heads is to be explained as parasitic on the other head.

The two tongues are united at their base 3 cm. in front of the epiglottis. Each tongue shows the characteristic dorsal eminence. The fungiform papillae are numerous and clearly defined. In a normal adult there are from twenty to thirty vallate papillae irregularly distributed over the posterior part of the dorsal eminence. On the right tongue, outer margin, there are nine vallate; in the inner margin seven; on the left tongue, outer margin, seven, inner margin four—a total of twenty-six in the two tongues. Unless there is a marked increase in the number of vallate papillae after birth, then these structures are not doubled, which constitutes an interesting exception and one difficult to explain.

The two brains are united in the region of the trapezoid and posterior medulla. When the occipital bones were removed, the dura mater was continuous over the cerebellar region and was very tough.

On one side there was a sac-like swelling, which suggested that one head only contained a brain, but this proved to be untrue as soon as it was cut open.

The falx cerebri was loosely attached in the longitudinal fissure of the right brain. In the left brain, it extended halfway to the corpus callosum.

The tentorium cerebelli of the two brains is connected by a strong sheet, which is much thickened about 25 mm. from the longitudinal fissure of the left brain. From this thickening to the same region in the right brain, the sheet becomes thinner. The left tentorium cerebelli is massive and solid, extending over the left surface of the cerebellum and crura as a greatly thickened tissue with massive $\frac{v}{x}$ fibers showing on the surface, which is in marked contrast to the delicacy of this same structure over the right brain, where no indications of thickenings are to be seen.

In removing the brains the dura mater over the right brain was found to be more delicate than over the left.

The more critically one studies these two brains, the more evident it becomes that the right one is not a complete duplicate of the left. In a general way it is smaller in the thickness of the cerebral hemispheres, in the conspicuousness of the temporal lobe, and in the detailed pattern of the gyri (fig. 2).

The median vertical surfaces of these brains bring out the difference more clearly. In the brain of the calf there is the gyrus cingulum just dorsal to the corpus callosum, a supracingular gyrus and a marginal gyrus. This marginal gyrus passes posteriorly into the occipital lobe; while the cingulum and supracingulum unite in the region of the genu of the corpus callosum to pass around to the hippocampal gyrus. The main variation in these gyri is found in the right half of the right brain where the supracingular gyrus projects 5 mm. into the median longitudinal fissure beyond the outer gyri. It is also much thicker where it usurps almost entirely the place of the cingulum, of which only a trace remains. These two gyri in the opposite half, and in both halves of the left brain, are similar in extent and contour.

The general pattern of the gyri and sulci of the cerebrum is quite variable in calves, at least in the ones used this year in class work in neurology. Without specifying the detailed variation of these structures, it can be said that there is a marked difference in this respect between the right and left brains. Each brain was compared with the brain from a normal calf, and it was easy to find one that exhibited a pattern of the gyri like the right brain and another like the left. The difference, then, is such as occurs in calves of separate parents.

There is also an interesting difference between the tracts of the olfactory and optic in the two brains. The piriform lobe is slightly irregular on the right half of the right brain. This irregularity consisting chiefly in the small size as compared with the other three olfactory lobes. In both brains, the two olfactory tracts on the surface (medius and intermedius) are prominent and of equal size. The intermedial tract just as it enters the piriform sends a small tract to the tuberculum olfactorium. Here all of the parts appear alike.

There is no apparent difference in the four eyes. Each is normal in size and external appearance. A well-formed optic nerve passed to the brain to cross in a definite chiasma. The median section of the brain revealed that the area of the section of the two chiasmata were very unequal, that of the left brain nearly twice as great. When the optic tracts are followed to their destination, those of the left brain continue as a broad, flat band to the superior colliculus, while the one on the right half of the right brain continues as a band for 15 mm. and then breaks up into two or three small bundles, the posterior one passes beneath some of the fibers of the cerebral peduncle, then coming to the surface and ending on the posterior margin on the superior colliculus, one strand of which enters the inferior colliculus. The optic tract of the opposite hemisphere is more like those in the left brain. Although each eye is normal, the optic nerve of one seems to have been disturbed during its growth in aberrant distribution of one of the optic tracts.

The four oculo-motor nerves are present, but slightly asymmetrical. A median section passing through the third ventricle leaves both nerves attached to the median half of each hemisphere. Each of the trochlear nerves was located in its normal relations, but the abducens could not be found.

As the two brains are joined from the region of the trapezoid to the posterior margin of the medulla, there is a great deal of confusion in these structures. In this region some injury was done to the right brain in dissection. The spinal cord was unmodified and more definitely united with the left brain. The fourth ventricle spreads out under both cerebella as a common cavity to the brachium pontis. The right wall of the medulla is much enlarged, having more than twice the thickness of the left, and from this region an irregular mass extends posteriorly about 2 cm. It is perfectly obvious that the right brain is dependent on the left, rather than each sharing equally a connection with the spinal cord. It would seem as if the right brain had failed to receive its full quota of embryonic nerve tissue, which cannot be said of the skulls.

The optic tract in the right brain certainly departs from the normal pattern—a condition which Miss Bishop says does not exist in the two-headed pig embryo. Whether it is proper to conclude that the gyri, especially the cingulum, also depart from the normal pattern is open to discussion. They are certainly not identical in the two brains.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 X-ray photograph of skulls and mandibles of two-headed calf. Note the similarity, even to minute details of tooth structure, mandibular canal, and sinuses which appear as light areas. Photographed by Dr. C. F. Potter.

2 The two brains with the remains of the dura mater showing in the longitudinal fissure and united over the cerebella. Notice that the detailed pattern of the gyri of the two brains is not alike. There is a posteriorly projecting mass beneath the right cerebellum, which together with other structural modifications suggests that the right brain is parasitic on the left. Photographed by Dr. C. F. Potter.



1



2

Resumen por el autor, A. R. Barnes.

La fascia pélvica.

El no haber reconocido la existencia de elementos estructurales en la fascia pélvica, cada uno de los cuales presenta diferencias en su desarrollo ha resultado en dos errores de descripción; el uso de los términos fascia visceral y parietal, es confuso y debe suprimirse. La descripción de las reflexiones de la fascia pélvica, que se consideran como una capa continua extendida sobre las paredes pélvicas y vísceras debe suplantarse por afirmaciones que indiquen donde se unen las unidades separadas de la fascia. La coalescencia de ciertas capas peritoneales contiguas en los fondos de saco prevesical y rectovesical y su transformación en fascia constituye una unidad estructural que está representada por las fascias umbilico-vesical y rectovesical. Las arterias umbilicales del embrión en las fases tempranas del desarrollo están rodeadas por una masa de tejido mesodérmico. Este tejido persiste en un forma condensada alrededor de estas estructuras, denominándose la vaina umbilico-vesical en el adulto. Del mismo modo, el tejido mesodérmico que envuelve a los vasos y nervios pélvicos, al recto y a la próstata persiste como en forma de vainas vasculares no organizadas que rodean a dichas estructuras. Otro grupo comprende las fascias relacionadas con los músculos, cuya extensión está determinada por la de los músculos con quienes están relacionadas. Con la excepción de la fascia de la superficie superior del músculo elevador del ano, estas fascias consisten simplemente del delgado perimio que se forma sobre los músculos en las demás regiones del cuerpo. El diafragma urogenital está formado por los ligamentos areuado y transverso pélvico y por un tabique fibromuscular bastante grueso. La superficie inferior del diafragma está envuelta por una fascia muy delgada.

THE PELVIC FASCIA

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EIGHT FIGURES (TWO PLATES)

INTRODUCTION

Much careful work has been done on the pelvic fascia. Almost every conceivable view of fascial arrangement has been advanced.

The following description is based on the development of the pelvic fascia, and has been found capable of demonstration in the class-room. The agreement between this description and the dissection has enabled the student to gain a clearer conception of pelvic fascia than has been possible from the accepted text-book description.

One of the greatest sources of confusion in the description of the pelvic fascia has been the attempt to show that it could be traced as a continuous layer over the entire pelvis and by some authors even over the perineum.

At the outset it is important to recognize that there are certain structural units of the pelvic fascia—units that can be accounted for by differences in development. The coalescence of certain contiguous layers of peritoneum and their subsequent replacement by connective-tissue lamina represents one type of fascia. The umbilicovesical and rectovesical fasciae belong to this first class. Secondly, we have the fasciae related to muscles; for example, the fascia covering the obturator internus, that covering the levator ani, and that of the perineal muscles. Lastly, we have that very considerable and much misunderstood mass of visceral mesodermal tissue surrounding the branches of the hypogastric arteries on their way to the viscera of the pelvis. If we keep these three structural units in mind, having as our object

the study of their arrangement, the question of continuity will become less important and will take care of itself.

This study has been confined to the male pelvis and perineum. Besides directing the dissection of some fifty-five pelvis in the laboratory, I have made twelve special dissections of cadavers, some injected with formalin, others in the usual way (equal parts of alcohol, glycerin, and phenol). Sagittal, coronal, and transverse sections of frozen cadavers have been freely used. Serial sections of the pelvis of human embryos of three, four, and five months were also studied. These sections were cut 20μ in thickness. Half the sections were stained in haematoxylin and eosin and the alternate sections in haematoxylin and picro-fuchsin.

THE UMBILICOVESICAL FASCIA

The extra peritoneal fatty tissue is commonly regarded as the only structure separating the fascia transversalis from the peritoneum. Over a triangular area, extending superiorly to the umbilicus and laterally to the obliterated umbilical arteries, two other structures intervene between the fascia transversalis and the peritoneum. These structures receive the name umbilico-vesical fascia and umbilico-vesical sheath in the adult.

Cuneo and Veau ('99) have shown the part played by the peritoneum in the origin of the umbilico-vesical fasciae. A short account of their work will serve to make clear its formation and arrangement. They have shown in their study of this region that the middle part of the embryonic bladder, together with the umbilical arteries, is at first placed in contact with, or partially imbedded in, the anterior abdominal wall. The bladder and arteries soon disengage themselves from this wall and invade the coelomic cavity. A section through a human embryo 45 mm. in length shows this latter condition (fig. 4). The bladder and umbilical arteries are seen surrounded by a mass of mesodermal tissue, called the allantoic sheath. They are covered by peritoneum except anteriorly where there is a connective-tissue stalk, the mesocyst, joining their mesodermal sheath to the anterior abdominal wall. This leaves a pair of culdesacs (fig. 4) anterior to the allantoic sheath separated by the mesocyst. This meso-

cyst is represented at an earlier stage by a mere strand of tissue. Then the culdesac on either side is gradually obliterated by the fusion of the peritoneal layer on the posterior surface of the anterior abdominal wall with that on the anterior surface of the allantoic sheath (fig. 5). The fused peritoneal layers persist as a thin but fairly strong fascial stratum called, in extra-uterine life, the umbilicovesical fascia. A study of my series of 45- and 80-mm. embryos confirms this view, and figures 4 and 5 illustrate the process described above.

The general contour of the umbilicovesical fascia is triangular (fig. 6). Accordingly, it has two surfaces—a posterior and an anterior—an apex, a base, and two borders.

The apex of the umbilicovesical fascia corresponds to the umbilicus. Leaving the umbilicus, the borders gradually diverge from the median plane. Each lateral border of the fascia is attached to the anterior surface of the peritoneum along a line lateral to the obliterated umbilical arteries (fig. 6), an attachment not hard to understand in the light of the process illustrated in figures 4 and 5. Delbet ('01) states that the fascia follows the umbilical arteries in their oblique course laterally and downward, and that it is attached to the entire anterior border of the great sciatic notch. This seems to me somewhat theoretical. I have found the fascia thinning out rapidly on its infero-lateral border and blending with the mesodermal tissue about the vessels of the bladder. Inferiorly and in the medial plane, we find the umbilicovesical fascia firmly adherent to the anterior surface of the bladder. It is possible in favorable cadavers to trace this fascia downward anterior to the bladder and demonstrate its attachments to the true ligaments of the bladder, the latter actually constituting the inferior attachments of the fascia in its medial portion (fig. 6). It is important to recognize that the base of the fascia is concave postero-inferiorly and that its concavity embraces the neck of the bladder.

UMBILICOVESICAL SHEATH

“The allantois arises as a tubular diverticulum of the posterior part of the yolk sac” (Lewis, '18), but subsequently is connected with the hind gut. McMurrich ('14) states that it

"pushes its way into the solid mass of mesoderm which forms the bellystalk." In an 80-mm. embryo we find the bladder and umbilical arteries imbedded in an extensive mass of mesodermal connective tissue (fig. 5), to which Cuneo and Veau ('99) have given the name allantoic sheath and for which a better name would be umbilicovesical sheath, inasmuch as the allantois is in the bellystalk and has no part in the formation of the bladder (Lewis, '18). Delbet ('01) states in this connection that the allantovesical sheath, preferably called umbilicovesical sheath, atrophies, becoming converted into a membrane stretching from the obliterated umbilical arteries laterally to the urachus superomedially, and the bladder inferomedially. This umbilicovesical sheath occupies a space bounded in front by the umbilicovesical fascia; posteriorly by the peritoneum and the rectovesical fascia (fig. 6).

Theoretically, the apex of the umbilicovesical sheath should reach the umbilicus in the adult. In practice one will hardly be able to trace it so high. It widens out as it descends, following for its outer borders the course of the obliterated umbilical arteries. Reaching the anterior angle of the flaccid bladder in the median plane, this membrane is continued over all the surfaces of the bladder forming its sheath. This covering has been erroneously described as coming from the fascia covering the upper surface of the levator ani. The lateral portion of the umbilicovesical sheath is continued downward past the lateral border of the bladder and blends immediately with the mesodermal tissue surrounding the vessels to and from the bladder.

It should be pointed out that the prostate is similarly developed in a mass of mesodermal tissue. This mesodermal tissue is later condensed about the prostate to form the prostatic sheath. This sheath joins the bladder sheath superiorly and blends with the superior surface of the urogenital diaphragm inferiorly. A small portion of the fascia on the superomedial surface of the levator ani is attached to the prostatic sheath laterally. Anteriorly, the sheath blends with the connective tissue of the preprostatic venous plexus, while posteriorly the sheath is joined by the rectovesical fascia.

THE RECTOVESICAL FASCIA

It has been pointed out how two layers of peritoneum in the prevesical culdesacs fused and were replaced by a fascia. A similar culdesac of peritoneum exists in the foetus between the bladder and seminal vesicles in front and the rectum behind. Usually a like culdesac exists between the bladder in front and the seminal vesicles posteriorly. Here, again, Cuneo and Veau ('99) have shown that these culdesacs are gradually obliterated by the fusion of the opposing peritoneal surfaces. The fused peritoneal layers persist as connective-tissue lamellae. The latter constitute the rectovesical fascia, one lamella separating the bladder and seminal vesicles, the other lamella separating the seminal vesicles from the rectum (fig. 7).

The rectovesical fascia has its superior attachment to the peritoneum as the latter leaves the rectum to pass on to the bladder (figs. 6 and 7). Extending downward between the bladder and rectum, the fascia has an inferior attachment to the sheath of the prostate gland immediately below the seminal vesicles. If this fascia is followed laterally, it will be found to blend very quickly with the mesodermal connective tissue about the vessels to and from the bladder. In figures 6 and 7 a fascia passing down in the interval between the bladder and seminal vesicle to attach to the prostatic sheath is clearly shown. I have not found this last lamina invariably present.

The rectovesical fascia is firmly attached superiorly to the peritoneum, so that an attempt to separate the two results in a tearing of the peritoneum. The peritoneum is usually slightly furrowed, due to this attachment.

SOME ANOMALIES IN THE DEVELOPMENT OF THE UMBILICO-VESICAL AND RECTOVESICAL FASCIAE

The peritoneal culdesacs, seen in the 45-mm. embryo, sometimes persist in the adult. In one dissection I found these peritoneal sacs persisting. They were located, as they are in the embryo, anterior to the umbilicovesical sheath, and each extended almost to the median line.

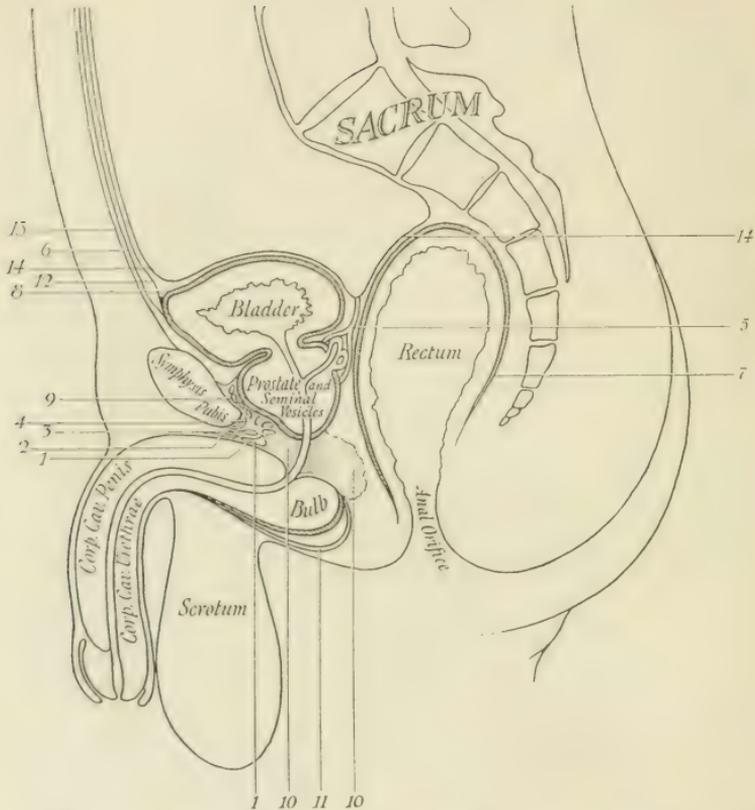


Fig. 1 Sagittal section of human pelvis through symphysis pubis (modified after Gray). Diagrammatic. 1, Ligamentum transversum pelvis and 2, its anterior lamina; 3, dorsal vein of the penis; 4, ligamentum arcuatum pubis; 5, rectovesical fascia; 6, umbilicovesical fascia; 7, condensed mesodermal tissue clothing rectum; 8, condensed mesodermal tissue of umbilicovesical (allantoic) sheath clothing the bladder; 9, puboprostatic ligament and venous plexus; 10, fibrous septa forming superior layer of urogenital diaphragm; 11, fascia covering bulbocavernosus muscle; 12, the umbilicovesical (allantoic) sheath; 13, fascia transversalis; 14, peritoneum.

In another dissection, the opposing peritoneal layers of the culdesacs had fused laterally in the neighborhood of the umbilical arteries. Failing to fuse medially, a pair of closed peritoneal pockets remained to remind one of the embryonic condition.

Similarly, the two peritoneal layers which fuse and later are replaced by the rectovesical fascia sometimes fail to unite. In one dissection I found such a condition in which the peritoneal pocket extended downward to the prostate, its lowest part separating the seminal vesicles and rectum.

Such anomalies as these substantiate the interpretation advanced above as to the origin of the umbilicovesical and rectovesical fascia, for the variations are exactly what one might predict, having the embryological condition in mind.

THE LEVATOR ANI AND ITS FASCIA

In a comparative study, Peter Thompson ('01) has found the iliococcygeus muscle arising from the iliopectineal line. The same muscle in man arises at various levels on the inner surface of the pelvic wall. Derry ('07) has found fibers of this in man reaching up into the neighborhood of the iliopectineal line—a condition the writer has encountered in two human pelves. Derry ('07) believes, therefore, that when the muscle falls short of this line, it is replaced by aponeurotic remains, an inference that my dissections repeatedly confirm. This aponeurosis is independent of the fascia covering the inner surface of the obturator internus muscle (fig. 2).

If the levator ani be dissected from below, it will be seen to arise from the inferolateral surface of this aponeurosis. Accordingly, as Derry ('07) has suggested, above the level of origin of the iliococcygeus muscle from its aponeurosis is an area of the pelvic wall clothed with two fascial elements, viz.: *a*) the aponeurosis of the iliococcygeus and, *b*) the thin fascia covering the obturator internus. This area is limited posteriorly by the anterior border of the greater sciatic foramen.

The upper surface of the levator ani is clothed by a distinct fascia usually well described. Delbet ('01) gives perhaps the

most exhaustive and precise account of its extent. The inferior surface of the muscle is clothed by a very delicate sheath, which, if called fascia, ought to be recognized as a thin perimysium. Superiorly, the portion of this perimysium related to the pubococcygeus muscle joins the aponeurosis of that muscle, while anteriorly the part of the perimysium related to the pubococcygeus muscle joins the pubic bone and its ascending ramus. Inferiorly the perimysium joins the anal fascia (fig. 3).

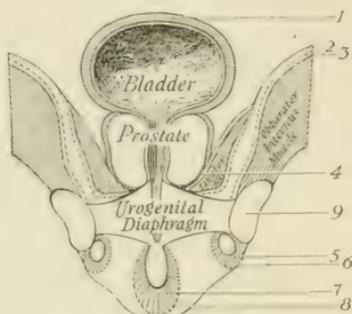


Fig. 2 Schematic drawing of coronal section of pelvis through prostate (modified after Cunningham). 1, bladder sheath; 2, aponeurosis of levatorani muscle; 3, perimysium covering inner surface of obturator internus muscle; 4, blending of fascia on upper surface of levator ani with sheath of prostate; 5, ischio-cavernosus muscle and, 6, its perimysium; 7, bulbocavernosus muscle and, 8, its perimysium; 9, descending ramus of pubis.

Two lines are frequently encountered on the upper surface of the levator ani. The more constant of these is the arcus tendineus fasciae pelvis. It stretches from the postero-inferior border of the pubic bone and symphysis to the ischial spine. The writer regards this as a thickening of the fascia which covers the upper surface of the levator ani muscle. It serves to fix the bladder and prostate to the wall and floor of the pelvis. Derry ('07) states here: "In this way the pubo-prostatic and lateral true ligaments of the bladder are formed." I believe his interpretation entirely correct. This line serves to join intimately the fascia of the superior surface of the levator ani with the bladder sheath and has been the basis for the erroneous

conception that the bladder is covered by a fascia formed by a reflection of the levator ani fascia.

Another line less frequently present is the arcus tendineus levatoris ani. It stretches across to the ischial spine from a point on the descending ramus of the pubis anterosuperior to the obturator canal. Text-books give this line as the origin of the iliococcygeal portion of the levator ani—a statement that

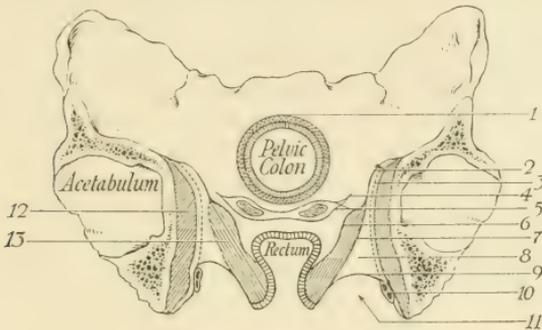


Fig. 3 Schematic drawing of coronal section of pelvis through acetabulum. (modified after Cunningham). 1, mesodermal tissue clothing colon; 2, aponeurosis of the levator ani muscle; 3, perimysium or fascia of obturator internus muscle; 4, lamina of rectovesical fascia splitting to enclose seminal vesicles and, 5, split portions enclosing seminal vesicles; 6, fascia on upper surface of levator ani; 7, perimysium, or fascia, on lower surface of levator ani; 8, supratagmental space; 9, lamina terminalis fossa ischioirectalis; 10, fascia lunata (here called Alcock's canal); 11, ischioirectal fossa; 12, obturator internus muscle; 13, levator ani muscle.

my dissections only partially bear out. In 40 per cent of cases examined with reference to this particular point, the levator ani had this origin. In 60 per cent of cases the arcus tendineus levatoris ani was absent and the levator ani took origin from the arcus tendineus fasciae pelvis.

The anterior fibers of the levator ani run above the urogenital diaphragm to obtain insertion into the perineal body. The fascia covering these fibers inferiorly is in contact with the superior surface of the urogenital diaphragm and is firmly attached to it.

The obturator fascia and the aponeurosis of the iliococcygeus join the periosteum just below the iliopectineal line. The iliac fascia also attaches to the periosteum at or just above the iliopectineal line. If we speak of the iliac fascia passing over the brim of the pelvis minor to become continuous with the obturator fascia and the aponeurosis of the iliococcygeus, we must recognize that the continuity is effected through their mutual attachment to the periosteum along the iliopectineal line.

The extent of the obturator fascia is determined by the extent of the obturator internus muscle, inasmuch as the fascia clothes its inner surface and has attachments at or a little beyond the attachments of the muscle. Wherever the obturator muscle is in contact with the urogenital diaphragm, its fascial covering is attached to the superior surface of the diaphragm.

It must be mentioned that the obturator fascia is ordinarily a very delicate membrane. It is, in the opinion of the writer, an ordinary perimysium for the obturator internus.

THE PERINEAL FASCIA

The first layer of fascia encountered in the ordinary dissection of the perineum is the superficial perineal fascia. It consists of two parts. The first part, the superficial portion, contains fat and is continuous with the superficial fascia over the thigh and gluteal region. The second layer, the deep portion of the superficial perineal fascia is commonly referred to as Colles' fascia, the extent of which is usually accurately described. The connective-tissue sheaths of the bulbocavernosus, superficial transverse and ischiocavernosus muscles blend with Colles' fascia on its deep surface. These muscle sheaths in turn blend with the deeper-lying inferior fascia of the urogenital diaphragm. It is through these muscle sheaths, then, that Colles' fascia becomes indirectly connected with the inferior fascia of the urogenital diaphragm—a point not made clear by text-books.

The urogenital diaphragm is ordinarily considered to consist of superior and inferior fascial layers, enclosing the deep transverse perineal muscle, the sphincter of the membranous urethra, together with branches of the pudendal artery and nerve. The

urogenital diaphragm will be better understood if dissected in accordance with the following description:

The urogenital diaphragm may be considered as composed of several units. Anteriorly, the first of these is the arcuate ligament—a structure well described in the best text-books. Next in order, posteriorly, comes the transverse ligament of the pelvis. It is separated from the arcuate ligament by the dorsal vein of the penis and, in turn serves to separate that vein from the dorsum of penis. The deep or posterior part of the transverse ligament blends with the fibrous tissues surrounding the preprostatic venous plexus. Still farther posteriorly is the third and most extensive element, a strong fibromuscular septum. Its thickness, vertically, varies from 4 to 8 mm. The urethra pierces this fibrous septum and branches of the pudendal vessels and nerves enter its posterior border and pass through it anteriorly. Posteriorly, it is attached to the perineal body; laterally, the attachment is to the inner surfaces of the descending rami, and superiorly it blends with the sheath of the prostate (figs. 1, 2, and 7).

Superficially, these three units are covered by the inferior fascial layer of the urogenital diaphragm which occupies the interval between the posterior border of the transverse ligament of the pelvis and the posterior border of the superficial transverse perineal muscle. The bulb of the penis rests upon the inferior surface of this fascia, covering the greater part of its central portion. The perimysium surrounding the bulbocavernosus muscle joins the inferior fascia, the latter gaining attachment laterally to the medial edges of the ischiopubic rami. Delbet ('01) has emphasized this attachment and remarks: "This layer (the inferior fascia of the urogenital diaphragm) is to my mind less an aponeurosis than a ligament of the bulb which it fixes so solidly." I do not share this belief. The inferior fascia is so thin that veins can be seen through it, and were it not for its intimate connection with firm deeper-lying structures its support of the bulb would be far from 'solid.' Deep to the inferior fascia are the vessels and nerves to the bulb, together with the deep transverse muscle and the sphincter of the membranous urethra (figs. 1, 2, and 7).

THE MESODERMAL SHEATHS OF THE PELVIC VESSELS, PELVIC COLON, AND RECTUM

The student is usually much confused when trying to dissect the tissue about the pelvic vessels in such way as to conform to accepted descriptions of the pelvic fascia. The same difficulty arises in the dissection of the tissues about the colon. In this connection, Delbet ('01) says: "Ombredanne ('00) has clearly shown that some analogous sheaths (analogous to the vesical sheath) exist about the principal viscera; they support the vessels which go to the organs, whence the name vascular lamina, which he has given them."

Sections of my embryos show that not only the pelvic vessels, but likewise the pelvic viscera to which they are distributed are developed in the midst of loose mesodermal tissue. In adult life we find these mesodermal sheaths condensed about these structures. Therefore, the connective-tissue coverings about the vessels and viscera which they supply are continuous with each other. The sheath covering the pelvic colon and rectum is very vascular and is so closely adherent to the gut wall that it cannot be dissected away as a continuous layer. Because of its vascularity and looseness, it does not resemble what we ordinarily call fascia. But we must recognize that such a sheath is present and must understand its continuity with the vascular sheaths. We can then avoid the false conception that the fascia related to the muscles of the pelvic floor is reflected to cover the rectum and lower portion of the pelvic colon.

The sheaths about the vessels and viscera are not condensed to compactness. But this will be recognized as the most practical arrangement, when it is recalled that the distention of the bladder and colon must be provided for. Fascia, inelastic as it is ordinarily considered to be, could hardly serve as a sheath for organs varying so much in size.

THE FASCIA LUNATA

The fascia lunata, a term introduced by Derry, is a sheath for the pudendal vessels and nerves. It can be demonstrated about these structures as they leave the pelvis through the greater sciatic foramen and may be traced to the urogenital diaphragm.

The fascia lunata has a slight attachment to the sacrospinous ligament, but it is very firmly attached to the inferomedial border of the anterior portion of the sacrotuberous ligament.

The fascia then comes into relation with the inferomedial surface of the obturator internus muscle. In this connection Smith ('07) says: "It (the fascia lunata) consists of an investment of fibrous tissue which has nothing to do with the sheath of the obturator internus muscle and is attached to the bony and ligamentous structures quite independently of it. It often happens (in the case of the human pelvis) that the fascia lunata becomes attached to the surface of the sheath of the obturator internus but this does not always happen."

The fact that we find quite as distinct a fascia lunata anteriorly and posteriorly where it has no relation to the obturator sheath supports Smith's ('07) view that it is not derived from the obturator sheath. However, all my dissections have shown the thin obturator sheath joining the fascia lunata (fig. 8), the latter gaining attachment to the sacrotuberous ligament, to the ischial tuberosity and to the ischiopubic ramus at a point about 1 cm. anterior to the fusion of the ischial with the pubic ramus.

The fascia lunata is commonly supposed to stop where the internal pudendal vessels and nerve enter the deep perineal pouch. It is no more deficient here than we saw it to be posteriorly as it emerged from between the greater and lesser sciatic ligaments. Instead of stopping, the sheath is broken up to be prolonged about the branches of the vessels and nerve in their passage through the perineum, thus constituting a considerable contribution to the urogenital diaphragm. Smith ('07) states that the fascia in the form of these sheaths actually becomes stronger because it affords an attachment to many fibers of the triangular ligament.

The portion of the fascia lunata which is related to the obturator internus muscle is commonly known as Alcock's canal. Smith ('07) has called attention to a septum passing from this portion of the fascia lunata to the perimysium on the lateral wall of the anal muscles. He calls this septum the lamina terminalis fossae ischiorectalis (fig. 8). I have been able to confirm the presence of this septum. It is important to note that this forms the roof of the ischiorectal fossa. This revises our view of the fossa which we have been accustomed to consider as extending upward to the junction of the fascia on the lateral surface of the levator ani muscle with the obturator fascia. The ischiorectal fossa is also limited anteriorly. It ends in several blind pockets filled with fat (fig. 8). These pockets may be regarded as being formed by a dipping downward of the lamina terminalis ischiorectalis. Smith ('07) points out that this arrangement keeps the ischiorectal fossa from extending forward above the urogenital diaphragm.

I am indebted to Dr. B. D. Myers for many helpful suggestions and for placing at my disposal abundant material for this investigation.

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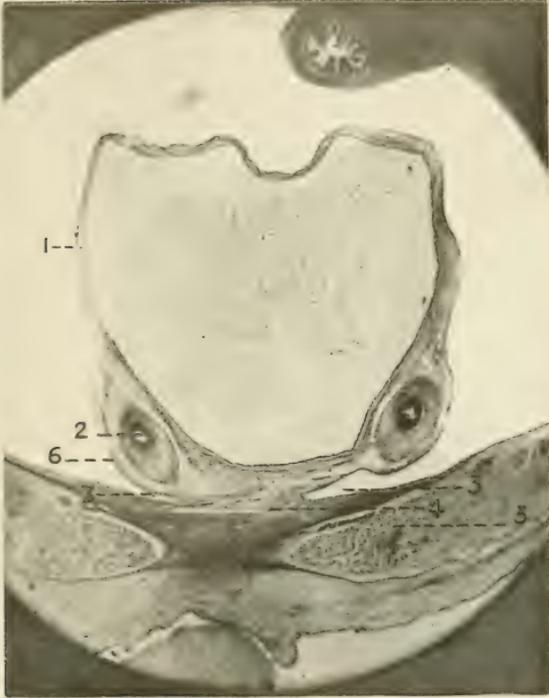
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PLATE 1

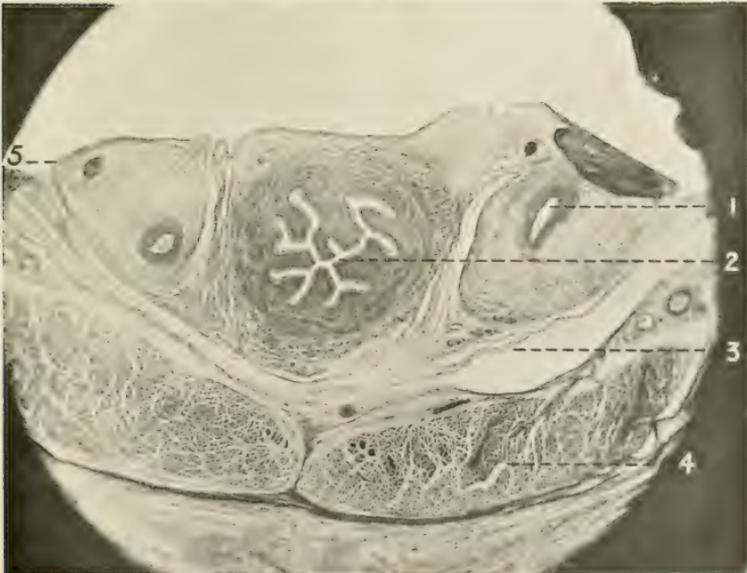
EXPLANATION OF FIGURES

4 Transverse section of 45-mm. embryo just above the symphysis pubis. Microphotograph. $\times 10$. 1, embryonic bladder and mesodermal connective tissue; 2, umbilical artery surrounded by mesodermal connective tissue, 3; peritoneal culdesacs separating the bladder from the rectus abdominis muscle; 4, the mesocyst; 5, rectus abdominis muscle; 6, peritoneum.

5 Transverse section through 80-mm. embryo just above the symphysis pubis. Microphotograph. $\times 10$. 1, umbilical artery surrounded by much mesodermal connective tissue; 2, embryonic bladder surrounded by mesodermal connective tissue; 3, line along which the opposing peritoneal surfaces fuse, obliterating the peritoneal culdesacs of figure 4; 4, rectus abdominis muscle; 5, peritoneum.



4



5

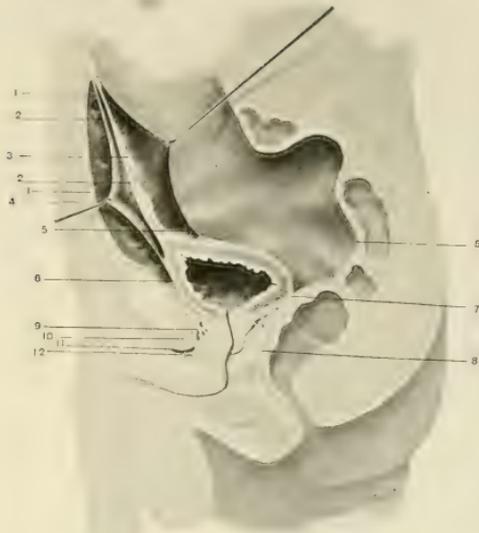
PLATE 2

EXPLANATION OF FIGURES

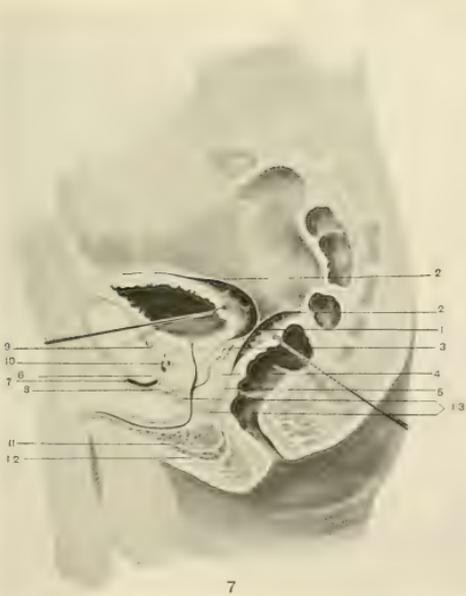
6 Sagittal section through symphysis pubis. 1, umbilico vesical fascia; 2, umbilicovesical (allantoic) sheath; 3, obliterated umbilical artery; 4, fusion of umbilicovesical fascia with anterior surface of peritoneum; 5, peritoneum; 6, attachment of umbilicovesical fascia to anterior true ligament of the bladder; 7, rectovesical fascia; 8, attachment of rectovesical fascia to capsule of prostate; 9, preprostatic venous plexus; 10, ligamentum arcuatum; 11, dorsal vein of penis; 12, ligamentum transversum pelvis.

7 Sagittal section through symphysis pubis. 1, rectovesical fascia; 2, peritoneum; 3, seminal vesicles; 4, prostate glands; 5, capsule of prostate; 6, ligamentum arcuatum; 7, dorsal vein of penis; 8, ligamentum transversum pelvis and anterior lamina; 9, retropubic fat; 10, preprostatic venous plexus; 11, capsule of bulb of penis; 12, perinysium of bulbocavernosus muscle; 13, fibrous septum making up a portion of superior layer of urogenital diaphragm.

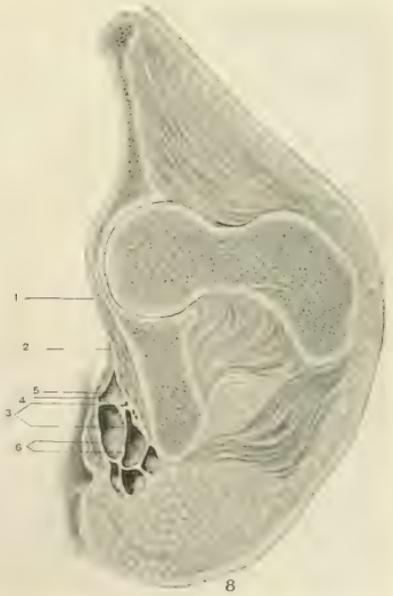
8 Coronal section through anterior portion of ischio-rectal fossa. 1, aponeurosis of levator ani muscle; 2, levator ani muscle; 3, fascia lunata; 4, lamina terminalis fossae ischio-rectalis; 5, supratragmental space; 6, blind pockets in anterior portion of ischio-rectal fossa (fat removed).



6



7



8

Resumen por G. Carl Huber, por el autor, Wilder G. Penfield.

El aparato de Golgi y su relación con el trofospongio de Holmgren en las células nerviosas. Comparación durante retispersión.

El autor revisa brevemente las investigaciones sobre el aparato de Golgi y el sistema de canales del trofospongio de Holmgren, prestando particular consideración a la idea, mantenida por ciertos autores, de que el aparato de Golgi y el trofospongio de Holmgren son la misma estructura en las células nerviosas, que se manifiestan de modo distinto según los métodos de fijación y tñido, que dan imágenes positivas y negativas de una misma estructura. Usando la misma célula y mediante métodos sucesivos de tñido, el autor ha observado que no se encuentran canales que correspondan con el aparato de Golgi demostrado previamente, y que durante la retispersión, el retículo de Golgi se desplaza hacia la periferia celular, mientras que la posición intracelular de los canales de Holmgren no se altera. Ambas estructuras pueden demostrarse sucesiva o simultáneamente en el citoplasma de una misma neurona.

Translation by José F. Nonidez
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THE GOLGI APPARATUS AND ITS RELATIONSHIP TO HOLMGREN'S TROPHOSPONGIUM IN NERVE CELLS. COMPARISON DURING RETISPERSION¹

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Beit Memorial Research Fellow

SEVEN FIGURES

INTRODUCTION

The intracellular relationship of the Golgi apparatus and Holmgren's trophospongium has been a subject of considerable dispute. Observations made by the writer while studying nerve cells in conditions of varying functional activity may throw some light on this problem. A preliminary brief review of the literature has been added, inasmuch as the work done on the comparison of the two structures has not been summarized in English and interesting contributions have been made since the excellent general reviews in German and Spanish by Duesberg and Cajal.

A reticular apparatus was first described by Camillo Golgi in 1898, in the cytoplasm of neurones in the central nervous system (21). In the same year Verati (44) described a similar structure in sympathetic ganglia. Outside of the nervous system, Pensa (40) was the first, demonstrating in the following year an internal reticulum in the cell cytoplasm of the suprarenals. Negri (37), also a pupil of Golgi, noted it in other ductless glands. After these pioneer observations, a large number of cytologists have observed the structure in cells of many types.

There has been considerable confusion as to the nomenclature of the structure in question. It has been variously called the internal reticular apparatus of Golgi (21), the endocellular reticular apparatus, the Binnemetz of Kopsch (33), the reticular

¹ From the Laboratory of Physiology, Oxford University. This communication formed part of a thesis accepted for the degree of B.Sc.

apparatus of Golgi-Holmgren (Cajal, 7), the canalicular apparatus (Bensley, 3), Golgi apparatus, etc.

Beginning in 1899, Holmgren (24) described an intracellular system of canals ramifying in the cytoplasm of neurones and certain other cells. He at once urged the identification of this canalicular structure, which he named 'trophospongium,' with the Golgi apparatus. Since this time the question of the identity of the two structures has been a subject of much discussion.

LITERATURE

Distribution of the Golgi apparatus in nature

A voluminous literature describing the apparatus in various types of cell has accumulated since Golgi made his original observations on spinal ganglion cells. The form of the apparatus varies somewhat from one type of cell to another, but common staining and developmental characteristics are exhibited throughout.

Cajal, in 1903, was the first² to demonstrate the Golgi apparatus in the nerve cells of invertebrates. His observations have been greatly extended for nervous and other tissue cells in all types of invertebrates by many workers, especially in the school of Nusbaum (38). Gatenby (19) states that "every sort of metazoan cell carefully examined, has been found to possess the typical apparatus" of Golgi.

Cajal (8) states that the reticular apparatus is well differentiated between the thirty-second and thirty-fourth hour of incubation in the germinal cells of the primitive streak (chick embryo). During ontological development, the reticulum and attraction sphere occupy the pole of the cell directed toward the external world, that is, the direction of cell migration or growth. According to Marcora (35), it is not until the tenth or twelfth day that the Golgi apparatus first appears in *adult* form about the cell nuclei of the more highly differentiated tissue. In cell division, Gatenby (20) has observed the Golgi apparatus (which is in the form of little rods lying on the archoplasm) to divide

² See Duesberg (16), p. 13.

itself equally between the two daughter cells. He concludes that "every mitosis or karyokinesis is also, as well, a dictyokinesis or nearly equal sorting out of Golgi rods between the daughter cells."

Bensley (3) described a typical intracellular reticulum in plant cells, staining with osmic acid, and watched it *intra vitam* break down from a living 'canalicular apparatus' to the 'multiply vacuolated' condition usually seen in the dead plant cell. This would seem to indicate that for plants, too, it may be possible, with improved technique, to demonstrate a reticular or canalicular cytoplasmic structure.

Thus the Golgi apparatus has been described as a cytoplasmic constituent of all types of cells in the animal kingdom, has been observed developing in the early stages of the embryo, and has been seen to grow and divide with cell multiplication.

Nature of the Golgi apparatus

The appearance of the structure varies somewhat from one type of cell to another, but in general it always occupies the cytoplasm of the cell and never encroaches upon the nucleus nor the cell periphery. In neurones it enters some distance into the dendrites. Its form is, in general, that of an irregular network.² In nerve cells the meshwork is typically studded with lacunae, which sometimes appear full of impregnable material and at other times empty, the boundaries *only* being outlined by the deposited silver or osmium.

With regard to the consistency of the apparatus, Cajal (8) has carried out some experiments which are of interest. In order to determine whether the 'materia argentofilia' was a liquid (and easily diffusible) substance or a semisolid substance not easily dislocated, he tore and crushed areas of spinal cord and brain. Upon subsequent microscopic examination, he found that at a tenth of a millimeter from the injured surface the Golgi apparatus

² Workers in the Institute of Nusbaum have shown (38) that in the ganglion cells of cephalopods the Golgi apparatus takes on the form of threads which rarely join or cross. Likewise, in gastropods, Crustacea, and some insects the apparatus departs somewhat from its usual reticular arrangement.

within the cells was quite normal. On the very borders of the lesion or at a short distance from it were cells whose internal reticulum was broken, but replaced by tiny globules distributed throughout the cell, quite in accordance with the usual structure of the reticulum. There was no seepage nor fusion into large drops of the component material of the reticulum. His conclusion was that the material of the Golgi apparatus was viscous or semisolid and incapable of diffusing irregularly through the interstices of the neurofibrillar skeleton.

Concerning the structure of the apparatus, Cajal's theory, which resembles that of Holmgren in some respects, is briefly as follows: As seen in its most highly developed state in nerve cells, but also in other cells of the body, it is made up essentially of two different factors, a stable system of communicating spaces, and "a finely granular, vacuolated substance composed of complex material, endowed with great chemical and physiological activity—a mixture of lipoids (substances reduced by osmic acid) and of cytoproteids (with special affinity for silver)." The quantity and chemical composition of this mixture vary within wide limits according to the functional activity of the cell. This complex chemical is organized, during life, into granules or microscopic 'protomeres' endowed with the capacity to grow and, in multiplying, to bring about or assist at the chemical synthesis indispensable to cellular life.

This theory is broad enough to explain the varying morphology of the Golgi apparatus at least in normal conditions, as demonstrated by the variety of methods in use.

Functional activity of the Golgi apparatus

That the apparatus plays some essential rôle in cell life can hardly be questioned, inasmuch as it has been found generally in the cells of all types of living organisms. Moreover, there exists a proportional relationship between the normal cellular activity and the size and complexity of the Golgi apparatus. For example, the most complete and complicated reticulum is found in adult nerve and muscle cells. The function of these two classes of cells is, of course, associated with a great discharge of energy.

In 1909, Golgi (23) observed in the mucous glands of the stomach of a frog the displacement and change in form of the reticular apparatus corresponding to the varying functional activity proper to the cells. Kolster (32) similarly noted dislocation and metamorphosis in the goblet cells of the stomach accompanying the formation of secretion, and Tello (43), in studying sebaceous and mammary glands, described two phases through which the Golgi apparatus passed in active cells: hypertrophy and dispersion into fragments or droplets.

Cajal (8), in the goblet cells of the intestine, showed that in the resting stage the Golgi apparatus was small. With the beginning of the formation of secretory products, the apparatus becomes much hypertrophied. Before discharge of the cell contents, the apparatus itself becomes fragmented and partially passes out with the secretion. He showed that in odontoblasts, and osteoblasts as well, there is hypertrophy of the reticulum with the onset of cell activity.

Basile (2) has made interesting observations on the renal epithelium of the remaining kidney in animals after unilateral nephrectomy. The apparatus, which normally is found between the nucleus and lumen of the tubule, becomes hypertrophied, passes down about the nucleus, and at the end of the twelfth day is found at the base of the cell. Addison (1) noted hypertrophy of the reticulum of the basophilic cells of the anterior lobe of the pituitary after castration.

Weigl (45), after considering the changes occurring in the Golgi apparatus of gland cells, concludes that no light is thrown upon the actual function of the structure in those cells. The changes, he supposes, are due to mechanical displacement. But Duesberg (15) has reported changes in staining reaction of the Golgi apparatus during cell secretion and absorption which, in his opinion, indicated a qualitative as well as a quantitative change.

As to the actual function of the Golgi apparatus in the cell, Nusbaum (38), in reviewing the work of Weigl and the other investigators in his institute, concludes that the nature of the activity of the apparatus is nutritive (also Cajal, 8) and that it shows, typically, phases of increase and decrease associated with

this activity. Duesberg (16) and Sjövall (42) are unable to reach any conclusions as to function, and agree with Golgi (23) that "Relativement à la signification de l'organe reticulaire en question, . . . il s'agit d'un probleme dont la solution reste encore à trouver."

Functional changes in the Golgi apparatus of *nerve cells* were reviewed in a previous communication⁴ (39), and a specific reaction of the apparatus in these cells to axone section was described. This reaction consists in a dispersion of the intact reticulum to the periphery of the cell, *retispersion*, which may or may not be followed by *retisolution*. The question arises as to whether this added phenomenon throws any light on the function of the Golgi apparatus.

Retispersion after axone section is usually associated with chromatolysis. The nucleus may therefore be found at the cell periphery as well as the reticulum. If the conclusion of Cajal is correct, that the Golgi apparatus is of a semisolid consistency, then in retispersion associated with chromatolysis the two cell constituents which are displaced to the periphery are of more or less rigid form. This might argue that they were passively displaced from the center of the cell, *without* necessarily signifying any alteration in the function of either structure.

From the form of the apparatus in nerve cells (a system of lacunae joined by canaliculi), its variations (sometimes appearing hollow and at others full of impregnable material), its resistance to alteration in abnormal conditions (such as tetanus and pathological states generally, or separation from its connecting neurones by decerebration or cord section), it may well be an endocellular storehouse or circulatory structure. That is, a structure of more or less rigid form, the lipid and proteid content of which varies during normal cellular activity. Its function is yet obscure.

⁴Since this was written I have had the opportunity of seeing a very interesting demonstration of changes in the Golgi apparatus of nerve cells in the spinal cords of rats following exposure to cold. Demonstration—Congrès de Physiol., Paris, July 16-20, 1920—by C. Da Fano. It is learned from this investigator that a preliminary note on the subject will appear shortly.

Methods of demonstration of the Golgi apparatus

The methods by which the internal apparatus of Golgi can be demonstrated in nerve cells have become numerous. Briefly they are:

1. Modern method of Golgi (22). Fixation in formol, alcohol, and arsenious acid, followed by impregnation with 10 per cent silver nitrate. Reduction in any developer. The sections are toned with gold chloride.

2. The Cajal method (8, 10). Fixation in formol and uranium nitrate; impregnation with silver nitrate, followed by reduction in a solution of formaldehyde, hydroquinone, and sodium sulphite. Da Fano (13, 14) has modified this procedure by the substitution of cobalt nitrate for the uranium nitrate in the fixative with good results.

3. Kopsch method (33). Immersion of fresh tissue in 2 per cent osmic acid for eight days.

4. Besta (5) stained the apparatus with thionin after fixation in formol and acetic aldehyde, and followed by mordantage in ammonium molybdate.

5. Sjövall fixes tissue in formalin followed by immersion in osmic acid.

In addition to the above well-known methods, the apparatus is occasionally stained by many of the more general methods or may be seen unstained against a darker background (12). It may be stained *intra vitam* with neutral red or janus green (18). It has been demonstrated to me by J. B. Gatenby in fresh unstained cells of the ovotestis of *Helix aspersa* when they were teased out in the tissue juice.

A description of the methods employed in this study appears below.

Mitochondria (chondriosomes) and Golgi apparatus

Most recent writers are agreed that mitochondria and Golgi apparatus are separate structures, although possibly having some genetic relationship (Duesberg, 17, Cajal, 8; Cowdry, 12; Gatenby, 18; Nusbaum, 38). On the other hand, Monti (36) has recently

maintained that in adult nerve cells the mitochondria and Golgi apparatus are one and that the apparent difference is due to the different technic employed. It is true that in procedures designed to demonstrate each structure, the other may occasionally appear as well, but this apparently is only an indication that the two structures have some common chemical constituents.

HOLMGREN'S TROPHOSPONGIUM

In 1899 Holmgren (24) drew attention to processes from outside entering the cytoplasm of the spinal ganglion cells of *Lophius piscatorius* and anastomosing there. In a postscript to this publication he cited Golgi's description of 'un fine apparato fibrillare' in the cytoplasm of the ganglion cells of mammals, and expressed the belief that the structure demonstrated by Golgi was only an intracellular part of the network of 'Saftkanälchen' which really communicated with the exterior.

In 1900 Holmgren (25) extended his observations to the spinal centers and ganglia of cats, rabbits, dogs, etc., using Carnoy's fluid principally for fixative, and iron hematoxylin or toluidin-erythrosin for stains. He showed that the 'Saftkanälchen,' or system of canaliculi, opened into the pericellular tissue spaces ('perizellulären Lymphspalten').

After these first descriptions he elaborated a somewhat complicated theory (26, 31) to explain variations in the appearance of the structure under consideration, which is briefly as follows: There is a network in the cytoplasm of certain cells to which he gave the name of 'trophospongium.' During cell activity, drops of liquid may be formed in the substance of the trophospongium thread. These drops coalesce, thus forming a canal within the thread, and this procedure may go far enough to dissolve the whole trophospongium, replacing it by trophospongium canals, the above-mentioned 'Saftkanälchen.'

In spinal ganglion cells he showed continuity between trophospongium and the enveloping subcapsular cells. In fact, according to Holmgren's view, the branching outgrowths of these enveloping cells or trophocytes, after entering the cytoplasm of the nerve cell, constitute the trophospongium. These ingrowths

possess the power of ameboid movements and their function is to take part in the nutrition of the neurone.

He proposed dividing all cells into two classes (26); *a*) a more highly differentiated class of cells all of which contain a trophospongium-canal system, and *b*) supporting cells, whose cytoplasm contains no trophospongium, but whose outgrowths, entering the cytoplasm of the cells of the first class, form a trophospongium there. Examples of this second class are subcapsular cells and neuroglia.

To sum up in Holmgren's own words (31, p. 135): "Summa summarum: ich glaube noch jetzt gültig Gründe zu haben . . . dass ein gewisser Inhalt des Netzes durch Umsetzungen in mehr oder weniger hohem Grade verflüssigt werden kann, wodurch Kanälchen entstehen, die durch azidophile Konturen, die den Resten der eigentlichen Fädchen entsprechen, abgegrenzt werden. An den Stellen des Netzes, wo die Dissolution zustande kommt, entstehen Kanälchen, die weiter sind als die übrigen Netzteile."

Numerous authors (Bethe, Cajal, Cowdry, et al.) have verified the existence of canals, *at least within the cell*.⁵

The question of the identity of the Golgi apparatus and Holmgren's trophospongium

In substantiation of his claim that trophospongium may be identified with the Golgi apparatus, at least in spinal ganglion cells, Holmgren presents in a recent article (31) excellent microphotographs of the Golgi apparatus blackened by the Kopsch method and, for comparison, iron-hematoxylin preparations in which the trophospongium canals stand out unstained in the ground cytoplasm. The general distribution of the two structures is the same and their form, although incompletely shown, is similar. Such appearances have led many authors to state that one structure is simply the negative picture of the other.

⁵ For an excellent summary of the work done on this point, see Duesberg (16), p. 47 et seq.

Such arguments are open to criticism; however. The two structures are being studied in cells of the same category, but *not in the same cell*. Also, even though they are frequently found occupying the same general location in the cell, their identity is not thereby established. Legendre (34) demonstrated similarity of general location for Golgi apparatus and Nissl granulations. But he did not thereby succeed in establishing that the two substances were identical.

Most workers have not been able to demonstrate extracellular connections for the trophospongium. In fact, Holmgren himself admits that all observers who have used only the osmic-acid or silver-impregnation methods, with the exception of Retzius and Smirnow, have concluded that the intracellular reticulum does not reach the surface of nerve cells. Sjövall (41), in ganglion cells, showed that it approached the subcapsular cells, but he could not establish true continuity.

In the opinion of Duesberg (16, 17), Nusbaum and his pupils, and also of Cajal (8), Holmgren, in maintaining the communication of the trophospongium with the exterior, has been confused by the appearance of exogenous processes which penetrate into (Duesberg, Nusbaum, Weigl), or make facets on (Cajal) the cell, without becoming continuous with the reticular apparatus.

Bensley holds that Holmgren's figures of intracellular nets in continuity with subcapsular cells stained by fuchsin do not prove continuity of structure, but more probably only a common affinity for the dye.

In a number of previous publications and in an admirable monograph ('15), summarizing the previous work, Cajal (8) has agreed that the trophospongium and Golgi apparatus in nerve cells were different representations of the same structure. The system of canals demonstrated by Holmgren, by Sjövall (41), and recently by Bensley (3), Cowdry (11, 12), et al., are produced, according to Cajal, not by a liquidation of the trophospongium, but by the fixatives used, partly or completely dissolving the reticulum. The occurrence of the canals would indicate failure of fixation of the reticulum or failure to stain it. The impregnation of the Golgi apparatus implies a fixation of the substance of

the reticulum and the deposition upon it of a metallic precipitate from the osmic acid or silver-nitrate solution.

Duesberg (16, p. 60), after a careful summary of the literature, concludes that the trophospongium, at least in nerve cells, cylindrical epithelium, and young oöcytes is identical with the Golgi apparatus in those cells. His conclusion was based on similarity of form and the changes undergone by the two structures at various ages of the animal. It should be noted here, however, that the development in the canalicular system has not been worked out as it has for the Golgi reticulum. Whether the structure appears as a set of canals or a compact network depends, in his opinion also, as in that of von Bergen, Bensley, Cowdry, et al., on the action of the reagents used.

The view taken by von Bergen (4) is somewhat different. He maintained that canals within the cytoplasm of ganglion cells are of two kinds. Type 1 resembles the Golgi apparatus in form and distribution and is really the negative picture of that structures. Canals of type 2 connect with the periphery of the cells, have no relation to the Golgi apparatus, frequently open into canals made by the microtome knife, and are in truth no more than artifacts. Canals of this type can never be stained, according to von Bergen. This theory has found few adherents. Holmgren's illustrations frequently show structures well stained which correspond to what von Bergen described as belonging to type 2.

Golgi and his school, on the other hand, refuse to consider the two structures as in any sense identical, although the former observed that some of the forms described by Holmgren are in some manner allied to the reticular apparatus. That the trophospongium and Golgi reticulum cannot be the same structure is, in the opinion of the Italian school, self-evident from the great difference in the form of the two structures. In agreement with the Spanish workers, they consider the reticular apparatus to be completely impregnated by the silver methods. The extracellular connections, therefore, as demonstrated by Holmgren, appear to the Italian school as additional evidence of the independence of the two structures.

Bethe (6) and Kopsch (33) likewise denied that the trophospongium is the same as the Golgi reticulum. The latter, using his entirely new but very simple osmic-acid technique, demonstrated an intracellular structure or 'Binnennetz' which corresponds very evidently with the Golgi reticulum and which, according to him, has no relation to trophospongium and does not show extracellular connections.

In considering the pros and cons of this much-debated question, it appears that the first group maintains that the Golgi apparatus and trophospongium-canal system are the same because of *similarity* of form and intracellular location. The larger part of this group deny the existence of extracellular prolongations of the canalicular structure (Cajal, Duesberg, Weigl, Bensley, Cowdry, et al.). The second group (Golgi and his pupils, Kopsch, et al.) deny that the two structures are the same because of *dissimilarity* of form. Much of the confusion arises from the variety of methods used, but also from the fact that Golgi apparatus and trophospongium have not been studied in the same cell carefully and that there has been no easily recognized alteration of one of these structures which could be evoked at will.

It is the object of this communication to draw the attention of cytologists to a method of successive as well as simultaneous demonstration of the Golgi apparatus and Holmgren trophospongium in the same cell, and to make a brief comparison of the two structures in neurone cytoplasm during retispersion subsequent to axone section.

METHOD

For this study the spinal cord and ganglia of cats were used, the Golgi apparatus being demonstrated by Cajal's formol-uranium-silver method, with certain minor modifications (39). After being stuck to the slide, the sections were counterstained by immersion in a dilute solution of Unna's polychrome-methylene-blue for from one to four hours. This was followed by passage through graded alcohols and differentiation in absolute alcohol. The sections were then mounted in Canada balsam,

and the result was a clear demonstration of Golgi apparatus and Nissl bodies, the latter appearing either green or blue, according to the intensity of silver impregnation in the cells. By this method the trophospongium canals may be demonstrated also. In some cells they will be found to stand out with great distinctness depending on the degree of differentiation. But I have found that these canals may be demonstrated more consistently and completely in the following way:

Drawings are made of the Golgi apparatus in certain selected cells (Cajal preparation). The cover-slip is then removed by immersion in xylol and the slide passed through downward graded alcohols to a 5 per cent solution of iron alum (Heidenhain), where it remains from twelve to twenty-four hours. This removes all silver from the cells as well as counterstain, and at the same time mordants the tissues for further staining. The sections are then treated as is usual in Heidenhain's iron-hematoxylin method. Great care is required to secure the proper amount of differentiation of the particular cells already drawn. At times it is necessary to mount the section in xylol and examine with high-power oil immersion, in order to be sure of the optimum differentiation of the Holmgren canals in the cell to be studied.

With the aid of the camera-lucida drawing previously made, it is easy to locate the same cell and to compare the two structures carefully. It is well to keep the nucleolus in focus during both examinations. Passing the sections up and down through the alcohol series must not be too abrupt, as otherwise a pericellular space may appear, especially in the spinal-cord sections. If a drawing was made of the canals demonstrated by the polychrome-methylene-blue in the original preparation, they will be found to coincide with those demonstrated now by iron hematoxylin in the same cell. Hematoxylin shows the canalicular structure usually in greater detail. It is evident that this staining is not influenced by the previous impregnation with silver, as there is no difference in the appearance of the peripheral as compared with the central cells. In the Cajal method the silver impregnation penetrates only a short distance below the surface of the block of tissue. No silver will be found in the central cells if the block is large.

If the section be left in the iron-alum solution for shorter periods of time, it is possible to mordant the tissue without removing the silver from the Golgi apparatus. The two structures then appear simultaneously in the same cell after staining with hematoxylin.

GOLGI APPARATUS AND HOLMGREN TROPHOSPONGIUM COMPARED DURING RETISPERSION

As mentioned above, after interruption of the axone there is a specific alteration in the distribution of the Golgi apparatus within the corresponding nerve cell, *retispersion* (Penfield, 39). As the name indicates, this alteration is a displacement of the net away from the center of the cell to the periphery. The axone base is also left clear of the apparatus (fig. 1). After this change there may or may not succeed a stage of dissolution of the Golgi apparatus, *retisolution* (fig. 2).

That the canals demonstrated by iron hematoxylin after removal of the silver as described above are really the same structure as that demonstrated by Holmgren, under the name of trophospongium, is apparent after comparison of his figures⁶ with those presented here (e.g., 25, p. 86, pls. I and II; 27, p. 324, pls. XXV and XXVI; 29, p. 380, fig. 2).

We may now examine the Golgi apparatus and trophospongium in the same cell under conditions which we know produce an alteration in the normal location of the former. The question arises whether or not in *retispersion* the Holmgren canals will likewise assume a peripheral distribution within the cell. If the two branching structures are identical, as certain authors maintain, one would expect to find both displaced to the cell periphery.

Such, however, is not the case. In figure 1 the Golgi apparatus (in black) was drawn with a camera lucida in a spinal ganglion cell impregnated with silver and without counterstain. *Reti-*

⁶ Whether or not these canals have any association with the neuroglial fibers which a number of authors have described penetrating the neurone body need not be discussed here further. It is also beyond the scope of this paper to discuss the possible relationship between the trophospongium and the so-called trophocyte. The attention will be confined to the cytoplasm of the nerve cells.



Fig. 1 Spinal ganglion cell of cat four days after section of the corresponding sciatic nerve. Retispersion evident. Successive staining method used (the Golgi apparatus and cell drawn from a Cajal silver preparation, the Holmgren canals are added after subsequent staining of the same cell with iron hematoxylin). Drawings made with Abbé camera lucida, Zeiss. Objective homog. immers. 1.5 mm., eye-piece no. 6 comp.

Fig. 2 Spinal ganglion cell of cat seven days after section of the corresponding sciatic nerve. Retispersion and retiresolution evident. Successive staining method as in figure 1.

spersion had begun throughout the ganglion, the sciatic nerve of that side having been cut four days previously. After removal of the silver, followed by staining with iron hematoxylin, the image of the same cell was superimposed upon the previous drawing by means of the camera lucida and the Holmgren canals drawn. It is apparent that the canals have not been forced to the cell periphery, as was the Golgi apparatus, under the influence of axone section, but have maintained the position occupied by them in normal cells (see also fig. 2).

If the canal system were the negative image of the Golgi apparatus, one would expect that drawings of a cell undergoing retispersion, treated in turn by the two methods described above, would show the same network, black in one drawing and unstained in the other, provided the focus of the microscope was the same in each case. On the contrary, the general distribution of the two structures appears to be quite different (compare fig. 3 with fig. 4). In figure 5, a photomicrograph, it is apparent that the Holmgren canals are not influenced by axone section, although the Golgi apparatus has undergone retispersion. The form of the Holmgren canal system differs from that of the Golgi apparatus. It is branching, often arranged concentrically. The canals are less tortuous, of greater diameter, and have a much more regular outline. The larger lacunae of the Golgi apparatus, joined by smaller tortuous threads, are never seen in the trophospongium system. The canals at times communicate with the pericellular space, but the Golgi apparatus never.

Therefore the form and distribution of the two structures in the same neurone are frequently entirely different. Also, they do not react to axone section in the same manner. The conclusion is evident that the Golgi apparatus and Holmgren trophospongium are not identical.

But what is the relationship of the two systems? This may be more easily studied in cells showing both simultaneously. Figure 6 is from a spinal ganglion of a cat seven days after section of the sciatic on that side. The Golgi apparatus, which appears to be undergoing retispersion and also retisolution, has been impregnated with silver by the Cajal method and the Holmgren canals

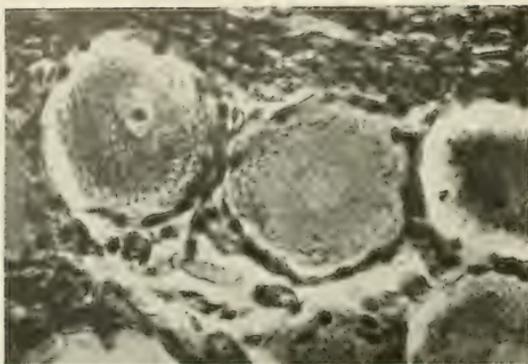
are simultaneously demonstrated by polychrome-methylene-blue. Nissl bodies are almost entirely absent from the cell except just about the nucleus. When the drawing was made the nucleolus was kept constantly in focus. The section of the cell drawn has therefore no greater thickness than that of the nucleolus. The



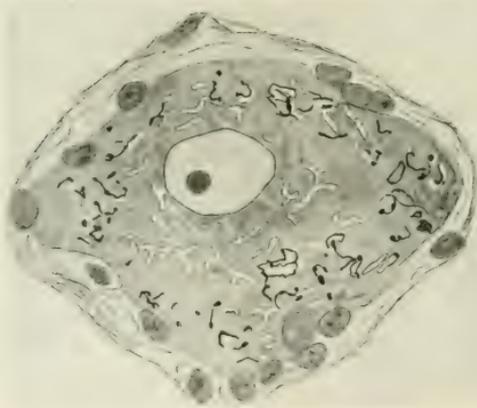
Fig. 3 Group of three cells from spinal ganglion of cat seventeen days after section of the posterior nerve roots. Retispersion and retiresolution evident. Cajal silver preparation. Object. homog. immers. 1.5 mm. eye-piece no. 1.

Fig. 4 Same cells and magnification as in figure 3. The silver has been removed and followed by iron hematoxylin. In this drawing, as in figure 3 also, the nucleus at its greatest width was kept in focus while each cell was being drawn.

same contrast in the general location and form of the two structures as was noted above is evident. But in some areas a part of the Golgi apparatus is seen to lie against the wall of a Holmgren canal and to follow its contour. Subsequent staining of the same cell with iron hematoxylin showed the same canals. It is, no



5



6

Fig. 5 Photomicrograph. Magnification of 900, Cajal silver impregnation with polychrome-methylene-blue counterstain. Cells of spinal ganglion of cat subsequent to axone section. Golgi apparatus dark. Holmgren canals unstained. In the less darkly shaded cell the Golgi apparatus is at the periphery in evident retispersion, while in the center of the cell may be seen Holmgren canals, faintly outlined in two concentric groups. The chromophil substance has largely disappeared from this cell. In the darker cell the presence of the blue-stained chromophil substance partially obscures the black Golgi apparatus at the periphery of the cell, but demonstrates the Holmgren canals both in the center and at the periphery with greater distinctness.

Fig. 6 Cell from spinal ganglion (fig. 2). Simultaneous staining method. Same magnification as figure 1.

doubt, such appearances which have led Holmgren to speak of canalization of the trophospongium. The part impregnated with silver in this section would, I presume, according to his interpretation, be that portion of the trophospongium wall not liquefied. Figure 7, a photomicrograph of normal cells, shows these two systems clearly and it is apparent that the Golgi apparatus frequently lies beside the unstained canals.

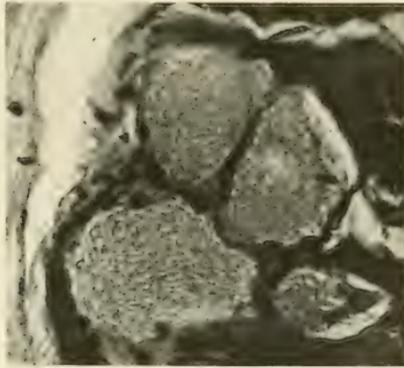


Fig. 7 Photomicrograph. Magnification of 900. Treated as in the successive staining method without, or with very little, removal of the silver from the Golgi apparatus. Cells of normal spinal ganglion of cat. In the lower large cell the relation of the Golgi apparatus, in black, to the Holmgren canals, unstained, is illustrated.

If one were to adopt the theory of von Bergen, on the other hand, that these canals are really artifacts or cracks in the cytoplasm, it would be sufficient to say that the crack tends to occur along the outline of the reticulum in certain cases. Subsequent staining of this cell with iron hematoxylin showed the same canals. In view of the excellent work of Holmgren and also the regular consistent appearance of the canals when the tissue is fixed without the slightest trace of shrinkage or distortion, it would seem extremely unlikely that these Holmgren canals are artifacts in any sense.

The above facts show that the Holmgren canals are not simply the negative picture of the Golgi apparatus. Fixation has been

in formol-uranium-nitrate solution which does not dissolve the Golgi apparatus. It is conceivable that if Carnoy's fluid, for example, were used for fixative, that the Golgi apparatus, after being dissolved or changed in some fashion, would then leave its negative image in the form of unstained canals. It does not seem likely, however, that these negatives would bear much resemblance to the canals of Holmgren. All reason for the negative-picture hypothesis disappears when the two structures are demonstrated simultaneously in the same normal cell (fig. 7) and are shown to react differently to abnormal conditions.

Occasionally, as noted above, I have seen canals and Golgi threads lying side by side. This may indicate, perhaps, a close association of function, but, inasmuch as we do not understand the function of either structure, the nature of their interrelation can only be conjectured.

SUMMARY

A short review has been made of the work which has been done, chiefly in continental laboratories, on the Golgi apparatus. It has been described as a cytoplasmic constituent of the cells of all animal organisms. It has the power of growth and has been followed through cell development and division. Its form is generally that of a network, but in certain of the lower invertebrates it appears as a thread or rod.

The trophospongium-canal system described in the cytoplasm of certain cells by Holmgren has long been urged as identical with the Golgi apparatus. Both systems find their most complex development in the cytoplasm of nerve cells.

Cajal, Duesberg, Bensley, Cowdry, Nusbaum, Weigl, and others have refused to accept the extracellular connections described by Holmgren. They have maintained or implied that the Golgi apparatus and Holmgren trophospongium were the same structure in nerve cells; that this structure appeared as a network which may be impregnated with silver or with osmic acid only after adequate fixation, and that, in case the fixative was inadequate or contained certain solvents, the structure appeared as an unstained canalicular system.

Golgi and his pupils, Kopsch, Bethe, and others, have never accepted the identity of the Golgi apparatus and Holmgren trophospongium, because of the difference in their form and also the extracellular connections of the canals described by Holmgren. On the contrary, those just mentioned above, who take a similar view with Cajal, find the form of the two structures similar and deny extracellular connections. Their general location within the normal cell is admitted to be the same.

The confusion seems to be due partly to the variety of methods of study, but also to the fact that comparison has been made in cells of the same type, but not the same cell, and there has been no means of inducing a specific change in either structure. It has been shown above that after the Golgi apparatus, as demonstrated by the Cajal method, has been studied in a certain cell, the silver may be removed and the trophospongium demonstrated in the same cell by iron hematoxylin; also that the two structures may be demonstrated simultaneously, as illustrated in the drawings and photomicrographs.

These methods provide a simple means of direct study and comparison. In the successive staining method no canals are found which correspond to the Golgi apparatus previously demonstrated. During retispersion (a specific alteration of the Golgi apparatus described in a previous publication) (39), the Golgi net is displaced to the cell periphery, while the intracellular location of the Holmgren canals is not altered.

CONCLUSIONS

That the Golgi apparatus is a universal cytoplasmic constituent has been established by a large number of investigators. In neurones the apparatus reacts to axone section in a specific manner. There is no similar response on the part of Holmgren's trophospongium. The two structures may be demonstrated independently in the cytoplasm of the same neurone, either successively or simultaneously.

The conclusion is evident. There cannot be a positive and a negative picture of the same structure, as has been widely maintained. They are separate structures demonstrable simultaneously.

Occasionally there appears a close anatomical relationship between parts of the Golgi apparatus and Holmgren's trophospongium which may indicate an intimate association of function. The methods described above provide a simple means of comparative study.

Further work upon the Holmgren canals is required to clearly demonstrate the developmental stages and, in fact, their existence antemortem.

In conclusion it is a pleasure to thank Professor Sherrington for his interest and suggestions. To Prof. Arthur Thomson I am indebted for helpful criticism, and to Mr. Chesterman, of the Anatomical Department, for the photomicrographs.

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Resumen por el autor, Paul H. Stevenson.

Sobre una anomalía poco común del peroneus tertius de un chino.

Una interesante anomalía del músculo peroneus tertius descubierta en las dos piernas de un chino constituye la base del presente trabajo. La anomalía consiste en la presencia de un peroneus tertius muy desarrollado, que se origina prácticamente en toda la extensión de la parte anterior de la superficie media del peroné. La inserción de este músculo tan desarrollado es próximamente la misma que la del músculo normal. El autor menciona brevemente la interpretación comunmente aceptada, que considera a este músculo "esencialmente humano" como una porción diferenciada del extensor largo de los dedos, y la consiguiente interpretación de las variaciones ordinarias de este músculo como simples variaciones en el grado de esta diferenciación. La anomalía discutida por el autor parece más bien representar una usurpación casi completa de la posición del extensor largo de los dedos, sin una correspondiente asimilación de la función de este último músculo. La presencia de una interesante deformidad en el calcáneo del pié derecho del mismo cuerpo se describe también en el presente trabajo. El autor plantea la cuestión de si el desarrollo exagerado del peroneus tertius y la tendencia aumentada resultante hacia la fuerte eversión del pié pueden o no considerarse como un factor en la producción de la deformidad descrita.

Translation by José F. Nonidez
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ON AN UNUSUAL ANOMALY OF THE PERONEUS TERTIUS IN A CHINESE

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ONE FIGURE

The peroneus tertius has long been recognized as a muscle appearing only in the human body,^{2, 4} and as such naturally attracts to itself a peculiar interest. It is usually considered to be a differentiated portion of the extensor digitorum longus,¹ and its variations therefore, which are very frequent, are commonly interpreted as mere variations in the degree of differentiation from this muscle.^{1, 2, 3, 7} The anomaly herewith presented however, seems to represent more than a simple variation in the degree of differentiation of one muscle from another, and differs sufficiently from those already described to make a note of it seem desirable.

The muscle in question was observed in a Chinese male, age 54 years, during the routine dissection of the lower extremity by first-year medical students. In both legs the muscle arose by a fleshy origin from the entire length of the anterior part of the medial surface of the fibula, with the exception of about 8 cm. at the extreme proximal end of the bone. The proximal 5 cm. of this latter space was occupied in turn by the diminutive origin of a very small extensor digitorum longus. The lower margin of the latter muscle and the upper margin of the anomalous peroneus tertius were both well rounded and distinctly formed, and separated by a distinct gap of approximately 3 cm. The peroneus tertius gave rise to a strong tendon which passed as a single structure beneath the transverse and cruciate ligaments of the ankle and divided into two separate tendons which became inserted into the base of the fifth metatarsal and the first phalanx of the fifth toe, respectively. The

small extensor digitorum longus gave rise to a very slender tendon which began at the upper margin of the peroneus tertius and passed down the leg and under the ligaments of the front of the ankle as a separate structure, subdividing on the dorsum

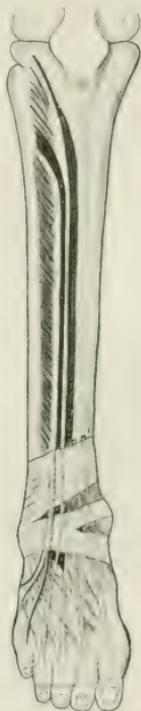


Fig. 1 Semidiagrammatic drawing, showing relations of anomalous peroneus tertius and extensor digitorum longus. Other anterior leg muscles represented by their cut tendons only.

of the foot into three smaller tendons having their insertions on the proximal phalanges of the fourth, third, and second toes, respectively (fig. 1). The nerve supply to both muscles was from branches of the deep peroneal nerve.

The general picture of this anomalous peroneus tertius did not suggest a mere variation in the degree of differentiation from the extensor digitorum longus. It implied, rather, that the peroneus tertius had almost completely usurped the position of latter muscle without assuming a proportionate share of its function. The question as to what effect this condition would have on the movements of the foot immediately presented itself.

It is interesting to note that the calcaneus in the right foot of this particular cadaver showed an unusual deformity. That portion of the bone bearing the main articular facet and receiving the greater part of the weight of the body through the talus, was not only depressed as if impacted into the cancellous portion of the bone beneath, but was tipped laterally on its anteroposterior axis, indicating that the force responsible for this impaction came while the foot was in a position of strong eversion.

Keith⁵ and Wood Jones⁶ have both emphasized the evolutionary importance of the peroneus tertius as providing the finishing touch in the eversion of the human foot. It might be considered an interesting question as to whether or not the development of this muscle beyond a certain point would be a factor in the production of injuries of the type found in this body.

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Resumen por el autor, Paul H. Stevenson.

El conducto biliar extra-hepático del camello.

El conducto biliar extra-hepático del camello (*Camelus bactrianus*) no presenta en su tamaño o forma indicio alguno de compensación por la falta de vejiga biliar de esta especie animal. En términos relativos, puede decirse que el conducto es estrecho y largo. Entra en el duodeno relativamente más lejos de esta parte que en el caso de las otras especies estudiadas en las cuales no existe vejiga biliar. El conducto pancreático se reúne con el biliar en el último tercio de su recorrido y a veces presenta un doble orificio de comunicación con el conducto biliar. El examen microscópico de la musculatura del extremo inferior del conducto ha demostrado la existencia de un aumento de las fibras musculares circulares que rodean al extremo duodenal del conducto biliar, indicando de modo muy evidente la presencia de un esfínter definido en este sitio. El autor ha llevado también a cabo observaciones sobre los sistemas extrahepáticos del caballo, ciervo, y rata albina, especies que carecen normalmente de vejiga biliar, y los resultados obtenidos se han reunido en un cuadro y también en una lámina de diagramas dibujados a escala con el propósito de que puedan ser comparados.

Translation by José F. Nonidez
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THE EXTRAHEPATIC BILIARY TRACT OF THE CAMEL

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TWO TEXT FIGURES AND ONE PLATE (FIGURES THREE TO SIX)

Judd,¹ in an experimental study of the effect of the removal of the gall-bladder in dogs, cats, and goats, observed that all the extrahepatic ducts dilated after cholecystectomy. Mann,² in a series of investigations on a larger number of animals, including some in which the gall-bladder is normally absent, endeavored to ascertain the relationship between the size and capacity of the extrahepatic ducts of animals in which the gall-bladder is normally absent to that of animals normally possessing this structure. He found no compensatory enlargement of the extrahepatic tracts of the horse, deer, pocket gopher, and the white rat.

The object of the present work is, 1) to corroborate the observations of Mann on the horse, deer, and rat, and, 2) to add to the literature on the subject of the extrahepatic biliary system of animals without a gall-bladder by a description of that of the camel. Inasmuch as the observations on the horse, deer, and the rat are entirely in accord with those published in the recent literature,² their mention in this paper will be confined to inclusion in the table of measurements and the plate of diagrams in which the biliary tracts of the various animals have been reduced to a common scale for the purpose of comparison of form and size.

The material for this study of the camel (*Camelus bactrianus*) consisted of the entire abdominal viscera of two camels, an adult and her calf of five months. Since a camel attains full growth at sixteen years,⁶ the small animal represents a stage of growth comparable to an eight months' human infant.

The data desired for the purpose of this work consisted of the following items: 1) the general relations of the biliary tract to the surrounding structures; 2) the length of the tract from the liver to the duodenum; 3) the diameter of the duct at several points along its course; 4) the distance from the pylorus to the opening of the common bile duct in the duodenum and, 5) the relation of the bile duct to the duct of the pancreas.

The distribution and arrangement of the muscle fibers, especially in the region of the orifice opening into the duodenum, were studied by microscopic methods.

DESCRIPTION

In general, the biliary tract, as well as the rest of the abdominal viscera of the camel, with the notable exception of the stomach and caecum which are of typical ruminant character, resembles that of the horse more closely than that of any other animal. The duct arises at the hilus of the liver, on the concave visceral surface, by the junction of several large intrahepatic bile ducts. It almost immediately enters that portion of the gastrohepatic omentum which embraces the upper flexure of the duodenum (ligamentum hepatoduodenale). In this mass of connective tissue the duct lies ventral to and in close connection with the hepatic artery and the large portal vein. The first part of the duct in the case of the young camel was inclined to be tortuous, but was quite straight in the case of the adult animal. In the last half of its course the hepatic duct lies on the head of the pancreas and is joined by the short pancreatic duct in the manner described below.

The free portion of the biliary tract, from the liver to the duodenal wall, measured 11 cm. in the adult camel. Entering the duodenal wall at an angle of about 60° , the duct immediately changes its course within the wall to one parallel to the long axis of the duodenum. The portion of the biliary tract within the wall of the duodenum, and spoken of in the table as the intramural portion, measured 3 cm. The entire length of the extrahepatic biliary tract in the adult camel was therefore 14 cm.

The opening into the duodenum took the form of a crescentic slit with the convexity directed downward, and the orifice was not easily discernible among the plicae cureulares of the intestinal mucosa.

The diameter of the duct proved to be remarkably uniform throughout its course. A very slight increase in size after the entrance of the pancreatic duct was noted, but was not in the least suggestive of a sacculation or of a dilatation as seen in this region in the tracts of some animals.⁷ In the adult camel the diameter of the upper part of the tract measured 0.9 cm., while below the entrance of the pancreatic duct this diameter was increased to 1.1 cm.

From the measurements made within the lumen of the duodenum, the orifice of the bile duct was found to be 40 cm. from the pyloric ring of the stomach.

At a point 2.5 cm. distant from its junction with the duodenal wall, the biliary tract is joined by a single large duct from the pancreas, and that part of the tract distal to this point acts as the common duct for the passage of both the pancreatic juice and the bile into the duodenum. The pancreatic duct approached the bile duct at an angle of about 70° , and in a direction contrary to that of the flow of bile.

Upon opening the bile duct opposite the entrance of the pancreatic duct, an interesting condition was found in the adult animal. The pancreatic orifices were two in number, although the free portion of the pancreatic duct was plainly single and showed no evidence of division before its juncture with the biliary tract. The two orifices opened one at either end of a slight oval depression. The upper margin of this depression formed a distinct flap, or valve, over the upper or smaller orifice, but the lower and larger of the two orifices was entirely open. In the case of the lower orifice there was apparently no valve-like structure present, unless the lateral walls of the oval depression approximated each other in life, forming a functional valve which would permit the escape of the pancreatic juice and at the same time prevent the passage of bile in the opposite direction.

It was largely to restudy this particular structure that the dissection of the second camel was undertaken. In the case of the second animal the pancreatic duct approached the biliary tract at more nearly a right angle, though still quite distinctly against the flow of bile. Upon opening the bile duct, some difficulty was at first encountered in locating the opening of the pancreatic duct. The orifice in this case was single and opened upon the floor of an oval depression, the lateral walls of which were closely approximated and reinforced by a sphincter-like arrangement of the mucosa. The protective mechanism against the passage of bile into the pancreas apparently consisted almost entirely of this redundancy of the mucous membrane surrounding the pancreatic duct orifice. Microscopic examination of that portion of the pancreatic duct entering the bile duct, in the case of the small camel, showed a few scattered muscle bundles, most of which assumed a longitudinal direction. No arrangement of muscle fibers into a circular sphincter-like band was recognizable.

As a possible explanation of the double orifice of the pancreatic duct in the adult camel, the question of a fusion of the accessory with the main pancreatic duct is suggested (fig. 1, A, B, C, and D). There was no separate accessory pancreatic duct present in either of the camels dissected in this study. The fact that there was, however, in both instances, a small contributing duct from the head of the pancreas joining the main pancreatic duct not far from its juncture with the bile duct, lends support to the suggestion of fusion as depicted in the diagrams. The presence of Brunner's glands in the submucosa of the biliary tract below the entrance of the pancreatic duct indicates the close developmental relationship between this portion of the tract and the duodenum, lending further support to the suggestion of fusion between the two pancreatic ducts which open separately into the corresponding portion of the duodenum in other animals.

The mucous membrane of the upper part of the biliary tract was perfectly smooth. Below the entrance of the pancreatic duct, however, the mucous membrane was thrown into minute longitudinal ridges. These ridges increased in size and number

toward the duodenum and finally merged with the plicae of the intestinal mucous membrane. Microscopic examination of this lower part of the tract showed Brunner's glands in the submucosa, as mentioned above.

Complete microscopic examination of the wall of the entire tract with special reference to the musculature was made. Muscle fibers, though present, were few and widely scattered

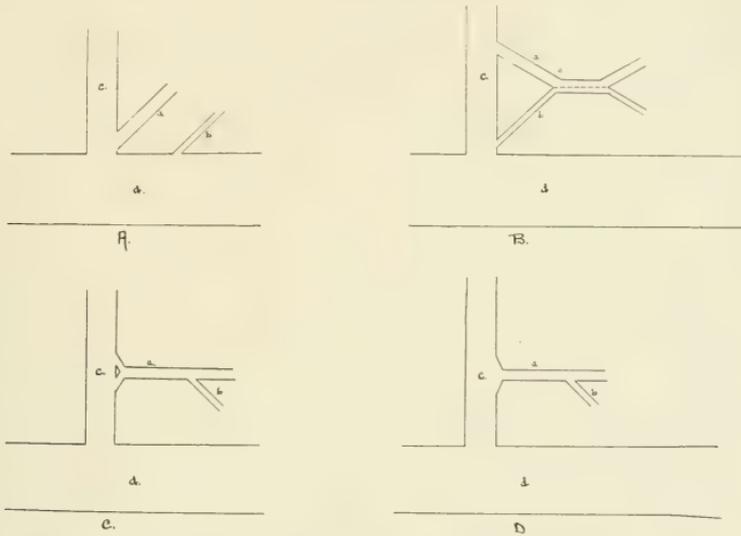


Fig. 1 Hypothetical development of the pancreatic duct in the camel. A. Biliary and pancreatic ducts as seen in the horse. *a*, main pancreatic duct; *b*, accessory pancreatic duct; *c*, biliary tract; *d*, duodenum. B. Theoretical stage. C. Biliary and pancreatic ducts as seen in camel no. 1. D. Biliary and pancreatic ducts as seen in camel no. 2.

in the upper part of the tract. They began to appear in distinct bundles, however, just above the entrance of the pancreatic duct. The direction followed by these bundles was in the main circular, but longitudinal and diagonal fibers were also present. In the wall of the duodenum there was a certain amount of blending of the muscle fibers of the bile tract with those of the intestine. This blending affected chiefly the longitudinal fibers.

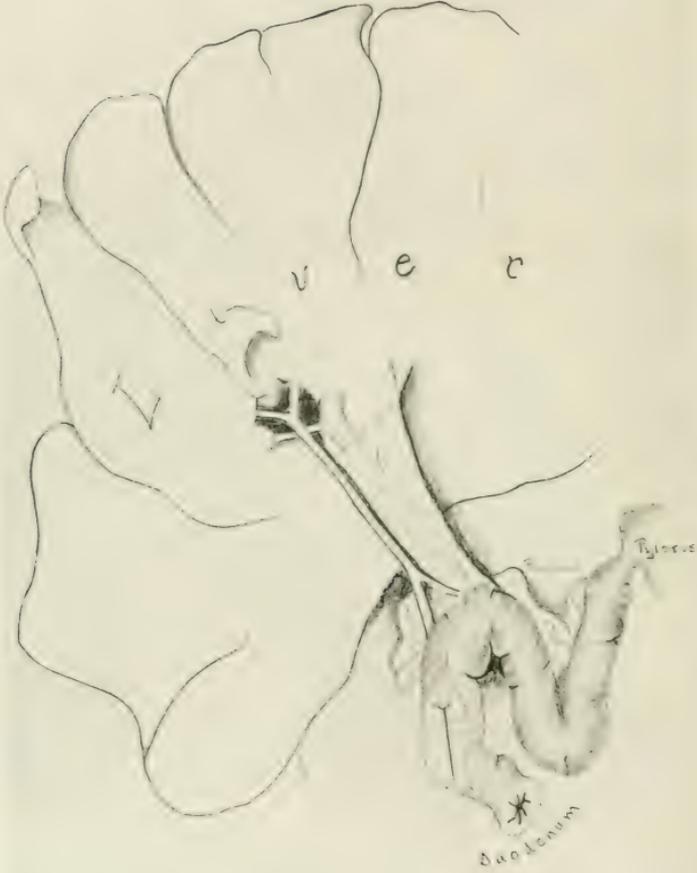


Fig. 2 The extrahepatic biliary tract of the camel

The circular fibers, which became increasingly prominent as the bile-duct orifice in the duodenum was approached, remained distinct and assumed a sphincter-like arrangement around the terminal portion of the duct.

The question of a sphincter around the end of the bile duct has long been an interesting and a somewhat debatable one. Comparative studies of the structure in various species have been made by Oddi,⁹ Hendrickson,¹⁰ Mann,³ and others. Oddi reported the absence of such a sphincter in the horse, and the subsequent inference of many was that animals lacking gall-bladders were also without this sphincter muscle at the end of the bile duct. Mann's investigation was undertaken to ascertain the presence or absence of this sphincter in animals without gall-bladders. In four species of animals without gall-bladders (the horse, deer, rat, and pocket gopher) he found "a definite arrangement of muscle fibers which might functionate as a sphincter in each species studied" (i.e., p. 360). Comparing these findings with those on a series of ten animals having gall-bladders, he found that "no difference could be observed in animals that have a gall-bladder when compared with those without one. There can be no doubt that anatomically some species of animals lacking a gall-bladder have an arrangement of muscle around the duodenal end of the bile duct which can act as a sphincter" (i.e., p. 360).

The present study shows that there is a similar arrangement of the circular muscle fibers around the duodenal end of the biliary tract of the camel to form a sphincter.

SUMMARY

The camel (*C. bactrianus*) belongs to that group of animals in which the gall-bladder is normally absent. The extrahepatic biliary system of the camel shows no suggestion either in size or shape of compensation for the lack of the gall-bladder. Expressed in relative terms, the extrahepatic biliary tract of the camel is long and narrow. It enters the duodenum relatively much farther from the pylorus than in the case of any other species thus far studied in which the gall-bladder is absent. The

pancreatic duct joins the bile duct in the last third of its course, and sometimes presents a double orifice. The circular muscle fibers become augmented in the intramural portion of the tract and are formed into a distinct sphincter-like arrangement surrounding the duodenal orifice of the duct.

Table of measurements

ANIMAL	LENGTH OF FREE PORTION	INTRA-MURAL PORTION	TOTAL LENGTH	DIAMETER	DISTANCE B-DUCT FROM PYLORUS
	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
Camel					
No. 1.....	11.0	3.0	14.0	0.7	40.0
No. 2.....	7.2	2.4	9.6	0.4	29.0
Horse.....	4.0	0.2	4.2	1.1	13.5
Deer.....	5.0	4.2	9.2	0.6	19.0
Rat.....	2.4		2.4	0.1	2.6

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PLATE 1

Schematic representation of relative sizes. Diagrams drawn to scale and conventionalized to correspond to similar diagrams by Mann.

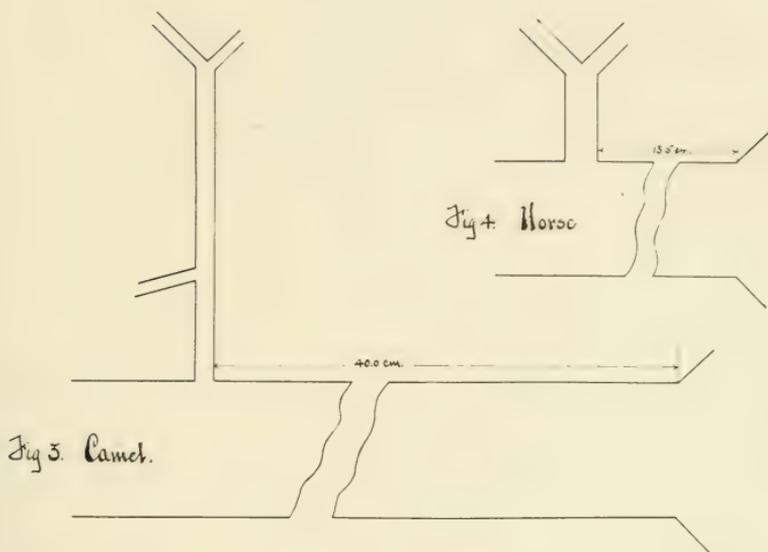


Fig. 3. Camel.

Fig. 4. Horse

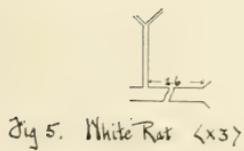


Fig. 5. White Rat (x3)

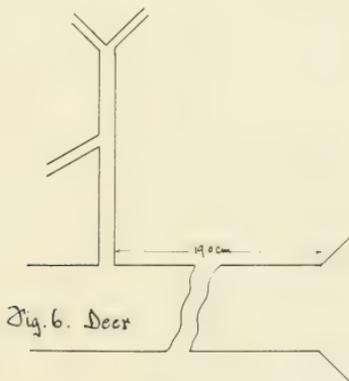


Fig. 6. Deer

Resumen por el autor, Berlin Berthold Nicholson.

Descripción de un método para exhibir y conservar los cuadros murales anatómicos.

El presente trabajo se ocupa de la descripción de un método efectivo de exhibir y conservar los cuadros murales anatómicos de gran tamaño.

Translation by José F. Nonidez
Cornell Medical College, New York

DESCRIPTION OF A METHOD FOR THE DISPLAY AND STORAGE OF ANATOMICAL CHARTS

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TWO FIGURES

The service of large colored charts in the teaching of anatomy is widely recognized. Often, however, the value of these teaching adjuncts is much impaired, because of an unsatisfactory method of storing and handling the charts. In order that they may be of greatest service to the student and instructor, the charts should always be readily accessible and conveniently arranged; otherwise, they will seldom be utilized by the average busy medical student.

The purpose of this paper is to describe a method of handling charts, which Dr. Wilmer Baker, now of Tulane University, and I devised, and which a three years' experience has proved to be highly satisfactory. The method consists of employing frames made from some light wood and swung by hinges in rows along the wall (fig. 1).

The structure of the frame can best be understood by referring to the detailed drawing (fig. 2). It is important to make the frame as light as possible and at the same time substantial and rigid. It must be thoroughly braced to prevent the outer end from sagging. It is likewise important to have the various parts firmly nailed or screwed together. Experience has shown that it is best to fasten the two front strips, which serve to hold the ends of the charts in place, with small screws; or better still, to pass the screws through a small metal plate the width of the wooden strip. This renders them less liable to split or to become pried off during the removal and replacement of charts. The frame should be the same length as the chart and about one-half of an inch taller than the chart itself.

The actual construction of the frame is relatively simple in the hands of any good carpenter. To simplify matters and for the sake of uniformity, it is best to carefully and accurately cut all of the parts in a miter-box at the outset. Then the putting together of the frames is readily and easily accomplished.



Fig. 1 Photograph showing the arrangement of the charts along the wall. It also shows the spacing between the charts and the manner in which they can be folded back. The taller frames are constructed in a manner similar to the longer ones, details of the latter being shown in figure 2. The first frame of each type shown in the tier has its front chart pulled out about 2 inches. (Charts here shown are made by Hoeft & Co., Philadelphia.)

Special care must be taken in cutting and nailing in the brace. It must be cut as long as possible and placed as shown in figure 2. It is important to thoroughly toe-nail it at both ends and on both sides, as well as to nail through the ends of the brace. Thoroughly seasoned lumber must be used if the frames are to stand up as they should.

The frames are best hung to 2 x 4 timbers bolted to the wall. These timbers should be so placed that the hinges will be as near as possible to the top and the bottom of the frame. A minimum stress is thus placed upon the frame and hinges. The frames must

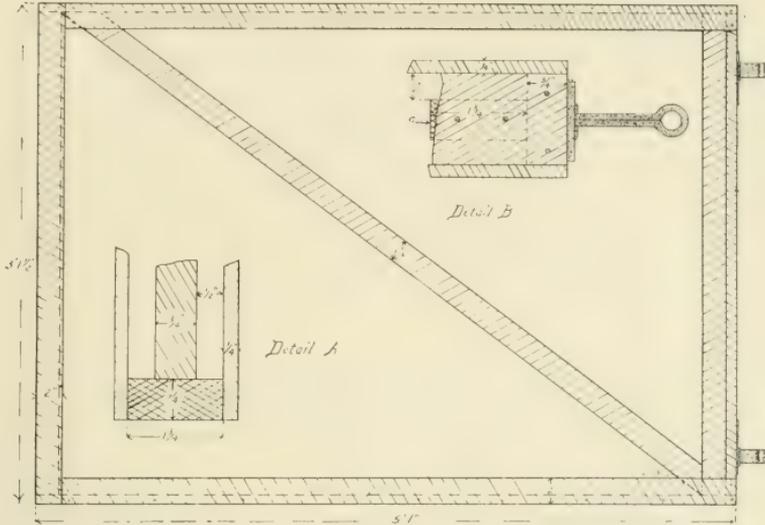


Fig. 2 Drawing showing side view of a frame. The broken lines show parts hidden from view. One half of hinge is shown, the other half bearing the post is fastened to the wall. Note the manner in which brace is placed. This position of brace offers the greatest possible strength.

Detail A. This shows the lower corner of the frame viewed from in front. Note the two 1/2-inch spaces for the charts, between which is a timber for the purpose of offering greater strength to the frame. The two lateral strips should be fastened with screws passed through a metal plate the length of the width of the strip.

Detail B. This shows the upper rear corner viewed from above. Note timber (a) which serves to offer rigidity and strength to the back of the frame; note also the manner in which the various parts serve to fasten the corner together.

be so placed that when they are swung toward the wall, the hinges will not be split off through the striking of the frame against the back part of its neighbor.

It is essential to use a hinge that has a long shank and one that is made in two parts, so that the frames can readily be re-

moved from their supports on the wall. With such a hinge the whole frame can be removed to another position and another readily put in its place, thus reducing to a minimum the handling of the charts themselves. Furthermore, when certain charts have served their purpose in a particular study, they can readily be removed for storage in the stock-room.

The method here described pertains only to large stiff charts for use in the laboratory. For handling and storage of flexible wall charts for general use in the lecture-room and laboratory the reader is referred to the method recently described by Reighard (*Anat. Rec.*, vol. 19, pp. 39-46, 1920).

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Resumen por el autor, N. W. Ingalls.

Una cámara estereoscópica simple.

Esta cámara no es sino la cámara fotomicrográfica modelo II de Bausch & Lomb modificada para el trabajo estereoscópico. En breves palabras, los cambios introducidos consisten: 1. En un nuevo ajuste de la cámara y los soportes angulares que la mantienen en una posición vertical para traer traer la lente y eje óptico en coincidencia con el centro de rotación de dicha posición. 2. En la elevación del centro de rotación a una altura conveniente para permitir un espacioso campo de acción. Esto pudo conseguirse mediante el empleo de un pequeño trozo de barra I, cuya superficie superior se inclina 8 grados para compensar el hecho de que el instrumento original solo permite movimiento lateral a la perpendicular. De este modo se puede colocar un objeto en el centro de rotación, enfocándole con la cámara vertical y haciendo exposiciones con la cámara inclinada de 3 a 5 grados a cada lado de la perpendicular. La tuerea opínza de que está provisto el instrumento original servirá para mantener la cámara en la posición deseada. Los objetos planos pueden fotografiarse con mayor ángulo que los objetos que poseen considerable profundidad. El ángulo disminuye también con el aumento de la distancia del objeto. Un ángulo demasiado grande tiende a deformar la imágen al exagerar la profundidad.

A SIMPLE STEREOSCOPIC CAMERA

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ONE FIGURE

The advantages of a stereoscopic photograph over the ordinary, single, flat photograph are so great and far-reaching that a brief account of the relatively simple apparatus necessary seems in order. For some little time the Anatomical Laboratory has been making extensive use of stereo-photographs, both as records and in routine teaching.

For purposes of record the method all but replaces the actual specimen, and for material which cannot for any reason be preserved, as embryological or histological material, the results are all that could be desired. The same applies also to objects, dissections, and the like, where, on account of their large size, mounting and preservation may not be practicable. The results with small specimens, such as one would ordinarily examine with the aid of a binocular, are especially gratifying when the photographs have been taken under a moderate magnification.

The use of stereophotographs as records of the human embryos in the department collection and also the methods of stereoprojection were demonstrated at the March meeting of the Anatomists in Philadelphia. The slides illustrating Doctor Todd's paper were all of bone specimens and ranged from reductions to enlargements: the slides of the author, representing embryological material, were mainly enlargements. The stereoprojection as shown at this meeting was the same as that in use in the department teaching. The same negatives furnish both lantern slides and prints, so that the student has the opportunity to review what he has seen on the screen by means of the mounted prints in the hand stereoscope.

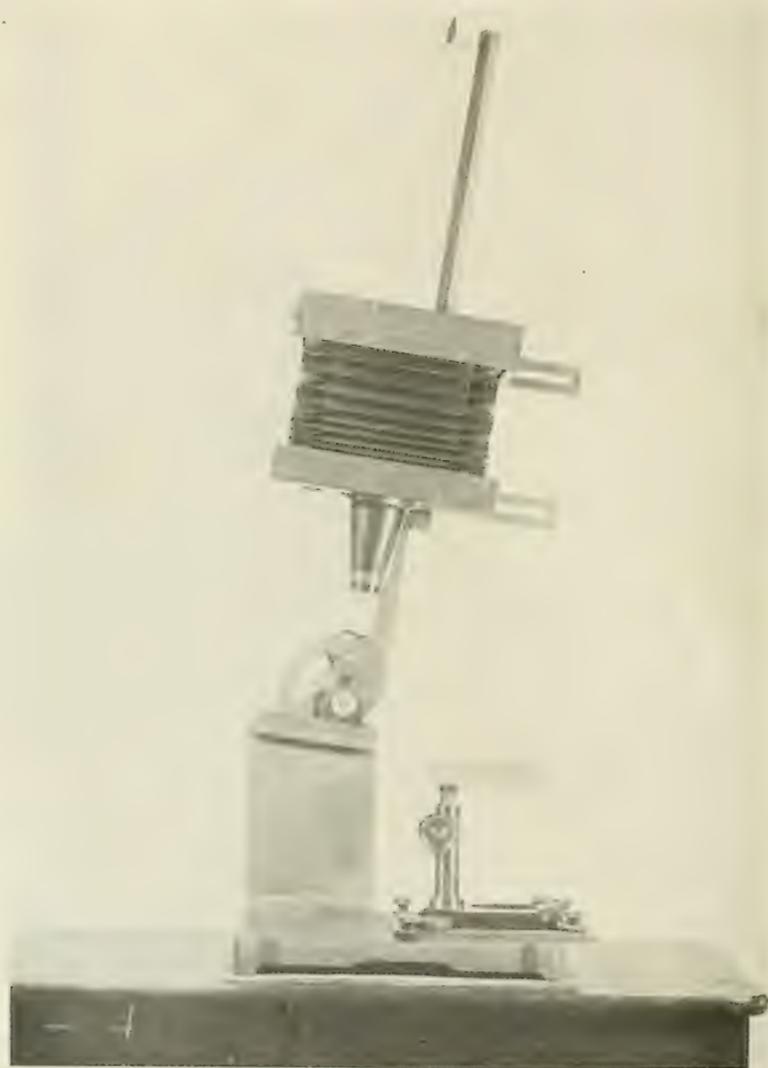


Figure 1

The stereocamera now in use is shown on the accompanying plate. This represents the Photomicrographic Camera Model H of Bausch & Lomb as modified by the author to meet the requirements of stereoscopic photography. Two alterations were necessary to adopt the camera to stereoscopic work. In the first place, the lens had to be brought over the center of rotation of the supporting arm, i. e., the optical axis of the camera must pass through the center of rotation. In its original form the camera box is placed on one side of the vertical support—on the right in the view represented—and some provision was made for lateral adjustment of the camera on this support. Since it was not possible to shift the lens over the center of rotation, it was necessary to remove the camera from the L-shaped brass straps to which it was attached, reverse these straps in the lateral supports, and replace the camera box. The pin with milled head which clamps the camera in lateral adjustments had to be removed and replaced in the brass strap nearer the angle, in order to allow the camera box to be pushed over the center of rotation. The manner in which the camera box has been readjusted may be seen from the original screw holes.

The second alteration serves a double purpose. It was necessary to raise the center of rotation to a sufficient distance above the base to provide working room, to accommodate objects of varying size and to permit their adjustment in a plane passing through the center of rotation. This was accomplished by removing the entire camera support from the flat base and placing it on top of a rather massive casting, I-shaped in section. It will be noticed that the top of this new support is higher at one end. The original mounting allows of movement only on one side of the perpendicular, so that by inclining the top of the new support the vertical axis of the instrument can be inclined about 10° on one side of the perpendicular and, with the old adjustment, nearly 90° in the opposite direction. The original clamp will hold the instrument in any desired position.

It is now possible to place an object in the center of rotation, which is in this case 230 mm. above the base, and to swing the camera to either side of the vertical about this same center.

Rotating the object instead of the camera has little if anything to recommend it, is obviously impracticable in most cases, and has the further disadvantage of changing the lights and shadows on the object.

The specimen to be stereoscoped is placed in the plane of the center of rotation and the camera focused as usual. Points exactly in the center will not change their position on the ground-glass when the camera is inclined to either side. Points at a higher or lower level or on the right or left of the axis of rotation will change their position. Since only a small linear portion of any object can lie in the axis, a very accurate adjustment is not called for. The small, remodeled dissecting microscope shown in the illustration is a convenient means of securing the important vertical adjustment of the object. To the upper end of the arm supporting the camera is attached a pointer which moves over a graduated arc on the wall close behind the instrument, so that the vertical position and the amount of inclination to either side can be readily determined. Much of our work has been done with an inclination of 5° or 6° on each side of the vertical, or a difference of 10° or 12° in the two positions of the camera. If the object is of considerable depth, a smaller total angle, 8° , 6° , or even 4° may be used, and the angle would naturally be reduced as the distance between the object and the lens increases. Too great an angle results in a distorted effect by exaggerating the depth.

The lens usually employed for objects of moderate size and also for enlargements is a 72-mm. tessar Ic. In the illustration this is mounted on an aluminum adapter 75-mm. long, to allow better illumination of the field. For large objects and with the camera at a greater distance, a 5 x 7 protar, series V, has given very satisfactory results.

Two exposures are made, on $3\frac{1}{2} \times 4\frac{1}{4}$ plates, at an equal inclination on either side of the vertical, 4° , 5° , or 6° if the total angle is to be 8° , 10° , or 12° . If a better view of the object can be obtained by inclining the camera, this altered position may be taken as the starting-point and the exposures made a given number of degrees to each side of this point. This is very convenient

in securing accurate views of objects which it may not be easy to adjust as a dorsal or ventral view of a small embryo. The finished prints are mounted as the negatives were taken, the right-hand view on the right and the left on the left and in such relation to each other that, as seen through the stereoscope, the two views fuse easily and completely. This is accomplished best by fixing one in place temporarily and making the adjustment with the other print while viewing both through the stereoscope. If the prints are reversed, the left on the right, the result is a reversal of the perspective, an intaglio instead of cameo, or vice versa.

The limitations of this instrument are due to the fact that the optical axis is only about 10 cm. in front of the vertical support. Consequently objects over 20 cm., 8 inches long, cannot be centered unless placed crosswise in the field.

Resumen por los autores, Waro Nakahara y James B. Murphy.

Sobre la naturaleza del llamado centro germinal en el tejido linfoide.

En una serie de estudios sobre la estimulación linfoide inducida artificialmente en varios animals han hallado los autores un marcado aumento en el número de figuras mitósicas en los centros germinales linfoides, el cual tiene lugar antes de la manifestación de la linfocitosis en la sangre, sin depender del método empleado para producir esta condición. Este paralelismo ofrece un apoyo experimental a la teoría original de Flemming, indicando la naturaleza linfoblástica de los llamados centros germinales de los órganos linfoides. Los métodos empleados para producir la estimulación linfoide fueron: 1) Inyección de tejido vivo homólogo seguido de inoculación de un tumor; 2) Exposición a una temperatura seca; 3) Exposición a una pequeña dosis de los rayos X, y 4). Exposición a una pequeña dosis de los rayos X seguida de inoculación de un tumor.

Translation by José F. Nonidez
Cornell Medical College, New York

ON THE NATURE OF THE SO-CALLED GERM CENTER IN LYMPHOID TISSUE

WARO NAKAHARA AND JAMES B. MURPHY

The Rockefeller Institute for Medical Research

TWO FIGURES

INTRODUCTION

The lymphopoietic function of the spleen and lymph-nodes is a well-known fact, but as to just how the production of new lymphocytes is brought about in these organs is still a matter of uncertainty. Flemming ('85) first called attention to the frequent occurrence of mitosis in the tissue of lymphoid organs, especially at a certain spot in the follicle. To this spot he gave the name 'germ center,' believing it to be a birth-place of new lymphocytes. The view has been traditionally followed in numerous texts, in which the lymphoblastic nature of the cells of the germ center is generally accepted and is based entirely on the interpretation of the static 'picture' as observed in preserved material. On account of the obvious limitations of this method of observation, the deductions made on it lack finality.

In the course of experiments on various types of general lymphoid reaction, we have collected observations which may serve to throw additional light on the process. A résumé of the observations and a brief discussion bearing on them are given in this paper in the hope of elucidating somewhat the point under consideration.

OBSERVATION

Lymphoid reaction induced by dry heat

It was reported by Murphy and Sturm ('19) that animals subjected to a small exposure of dry heat (using an electric heating lamp as the source of heat) show, following a sharp initial fall, a

rise of circulating lymphocytes often amounting to more than 200 to 300 per cent above the normal. Polymorphonuclear leucocytes participate in the fall, but they recover their normal level slowly and never rise above it.

Histological examination (Nakahara, '19) of the spleen and lymph-nodes of animals carried as a parallel to the above experiment showed that these organs, immediately after the heat treatment, contain numerous necrotic cells. The dead cells were present abundantly in the nodules and in the pulp of spleen, cortex, and lymph-cords of nodes. The cells of the germ centers were apparently unaffected, but cell division was totally suppressed.

It was also observed at about forty-eight hours after the treatment that, along with an elimination of the necrotic cells, there was an abnormally large number of mitotic figures present in the germ centers, and this hyperactivity of the centers continued for several days.

Lymphoid reaction in immunity to transplanted cancer induced by an injection of homologous living tissue

Mice, as is well known, can be made relatively resistant to transplanted cancer by injecting them with an emulsion of homologous living tissue ten days before inoculating the cancer grafts. Murphy and Morton ('15) found that the lymphocytic elements of the blood showed an active increase during this process, while polymorphonuclear cells were not appreciably affected. No change in the lymphocytes occurred in the resistant mice until the cancer graft was introduced, when an immediate and sharp rise took place in these cells. In the majority of animals the increase was 100 to 200 per cent above the normal and endured about two weeks.

The histological study (Murphy and Nakahara, '20) made of preserved material from the inoculated and resistant mice showed in the germ centers of the lymphoid organs increase in mitotic figures, reaching a maximum about five days after the immunizing injection and followed by a fall, the normal being regained by the tenth day. The resistant mice when inoculated with a cancer

graft ten days after the tissue injection show a second great increase in the mitotic figures, particularly of the germ centers. Judging from the number of mitosis, this second stimulation is more intense than the first.

Lymphoid reaction induced by small doses of x-rays

The selective action of X-rays on the lymphoid tissue has been recognized since the work of Heineke ('05). It was shown in this laboratory that while large doses of x-ray are destructive to, small doses bring about an increase in the lymphoid cells (Murphy and Morton, '15). In the case of rabbits thus treated, the number of circulating lymphocytes rose often in the course of one week (Thomas, Taylor, and Witherbee, '19) 100 per cent above the normal.

Histologically (Nakahara, '19) in these animals no appreciable destruction of cellular elements of spleen and lymph-nodes was observed; nevertheless the mitotic figures increased gradually in the germ centers, the height being reached in a few days after the treatment, and the increase persisted for a period of about two weeks.

Lymphoid reaction in immunity to transplanted cancer induced by small dose of x-rays in mice

Further, it was noted that in mice a certain small dose of x-rays of low penetration induces a stimulation of the lymphoid tissue (Nakahara and Murphy '20) and a regular and considerable increase in the number of mitotic figures of the germ centers takes place from twenty-four hours to four days after the treatment.

When cancer grafts were inoculated in these mice seven days after the x-ray treatment, an appreciable increase in resistance as compared to the normal untreated mice was detected (Nakahara and Murphy, '21). The blood picture of the mice, thus rendered comparatively more resistant, showed no constant change following the x-ray treatment, but the lymphocytes were found to rise 100 per cent or more above the normal within two weeks after can-

cer-graft inoculation (Nakahara and Murphy, '21). As in other varieties of cancer immunity, the polymorphonuclear leucocytes showed no appreciable change.

There was some variation observed in the histological appearance of the lymphoid organs of mice first x-rayed and inoculated with cancer grafts seven days afterward, but as a rule a profound acceleration of the rate of cell division was present in the lymphoid tissue, and especially in the area of germ centers (Nakahara and Murphy, '21).

DISCUSSION

The material which supplied the above data was studied primarily from the point of view of our interest in the relationship between the lymphoid cells and resistance to transplanted cancer in mice. In cancer immunity, induced by the usual means, namely, by the injection of living homologous tissue, it was noted that a marked increase in the circulating lymphocytes occurs, with which is associated an increase of cell multiplication by mitosis in the germ centers of the lymphoid organs. This relationship between the resistance to cancer inoculation and lymphocytosis was observed, regardless of means by which the lymphocytosis is induced. The rise of the lymphocytes of the blood is invariably preceded by an increased proliferative cellular activity in the germ centers. These facts suggest that lymphoid germ centers are really made up of lymphoblastic tissue, and are among the sources of lymphocytes. The cells comprising the so-called germ centers and the lymphocytes making up the nodule of lymphoid organs are often stated to differ morphologically, but in reality there is no criterion which serves actually to distinguish one from the other.

The fact that phagocytosis sometimes occurs in the area of germ centers and is absent from the lymph-nodules may be urged as evidence that the cells of the germ center are not lymphoid in nature, as lymphocytes are non-phagocytic. Such a deduction would seem too dogmatic, since embryonic mesenchymal cells, from which lymphoid tissue develops, are admittedly phagocytic. Moreover, it would be difficult to decide whether the large cells containing ingested cell fragments (Flemming's 'stainable particles')

really originate from the cells indigenous to germ centers or come from without. It is well known, also, that new lymphocytes are produced by a division of a preexisting one, which is capable of mitosis. This obviously cannot be considered as the only possible method of lymphocytic proliferation, as, embryologically, it is conceivable that cells with the potentiality of developing into lymphocytes may coexist in the adult along with the already differentiated lymphocytes.

SUMMARY

Several kinds of lymphoid reactions are described in which stimulation of the cell division of the germ centers of lymphoid organs preceded lymphocytosis in the blood. The association of the two processes described provides evidence that the lymphoid germ centers are in fact sources of blood lymphocytes, as the name indicates.

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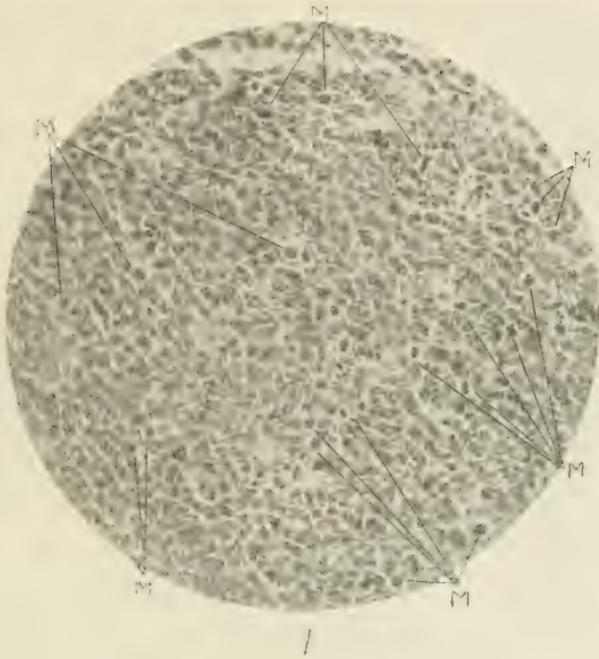
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PLATE 1

EXPLANATION OF FIGURES

1 A germ center of lymph-node of mouse treated with a small dose of x-rays of low penetration. Note the hyperactivity in cell proliferation as indicated by an unusually large number of mitotic figures (*M*). The majority of mice thus treated are potentially more resistant to transplanted cancer than untreated mice, and show, upon cancer inoculation, a marked increase in the number of circulating lymphocytes. $\times 480$.

2 An inactive germ center of lymph-node of mouse. Note the absence of mitotic figures. $\times 480$.



Resumen por el autor, C. D. Fife.

Ausencia de la carótida común.

En todas las formas esta condición es muy rara, debiéndose a una interrupción en el tercer arco aórtico y a la persistencia de la raíz aórtica dorsal entre los arcos tercero y cuarto. Este parte de la raíz dorsal aórtica es el conducto de Botal, que constituye un vaso funcional en muchos reptiles, especialmente manifiesto en *Sphenodon*. En el caso estudiado por el autor, el de un varón negro, falta la carótida común derecha, existiendo además un arco aórtico derecho; y la subelavía izquierda es del tipo normal arqueado, estando formada su porción proximal por la aorta dorsal izquierda más allá del cuarto arco, la cual recibe el conducto arterial. La disposición particular de las ramas del arco de la aorta es la consecuencia de la retención del cuarto arco derecho en vez del izquierdo. La rareza de la anomalía de la carótida es una expresión de la gran constancia con que la aorta dorsal se interrumpe entre el tercer y cuarto arco. La interrupción es muy antigua, estando ya bien establecida en los reptiles. También existía en el caso estudiado una vena cava superior izquierda que carecía de relación alguna con la derecha.

Translation by José F. Nonidez
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ABSENCE OF THE COMMON CAROTID

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THREE TEXT FIGURES

Absence of the common carotid is one of the rarest of the many anomalies which take their origin in the varying transformations of the aortic arches. Combined, as it is here, with other cardiac anomalies, it places the heart in question in a rather unique position.

On account of the rarity of the conditions found here and also because of the embryological interest which attaches to them, I have undertaken at Doctor Ingall's suggestion a brief account of the arterial anomalies and of their developmental significance.

The specimen, T. 71 of the teratological collection, was found in a dissecting-room subject, body no. 662, a male negro between thirty-five and forty years of age. Upon opening the thorax the heart was found in approximately its normal position. The aorta was directed upward and somewhat to the right along the right side of the trachea and oesophagus, the summit of the arch being just below the level of the disc between the first and second thoracic vertebrae. It then descended very obliquely toward the left, reaching its normal position on the left side about the level of the seventh vertebra. Corresponding with the oblique course of the aorta across the vertebral column, there was noticed a faint depression on the column extending from the second to the seventh vertebrae. Between the second and fifth vertebrae the oesophagus was inaccessible from the right side.

The branches of this right aortic arch are as follows (fig. 1): The first branch is the left common carotid which takes origin from ventromesial aspect of the first part of the arch close to the trachea and about 3 inches above the aortic ring. Next in order,



Fig. 1 Heart and great vessels, T. 71 of the teratological collection. The aorta and left cava have been displaced somewhat to show the arrangement of the vessels. Description in text.

and close together, near the summit of the arch, are the right external and internal carotids, the external almost immediately in front of the internal. While these two vessels are quite close together, their origins from the aorta are quite separate and distinct. The fourth branch is the right subelavian-not far from the carotids, while the fifth and last vessel, the left subelavian, arises $2\frac{1}{2}$ inches beyond the right from the ventromesial wall of the last part of the arch. This last-mentioned branch of the aorta presents at its origin a definite bulbous enlargement such as is usually found in this type of left subelavian. This enlargement measures approximately $\frac{1}{2}$ inch in diameter and from its apex the ligamentum arteriosum passes to join the pulmonary artery at the point of bifurcation. A very similar arrangement of the branches of the arch may be noted in the case of double aortic arch of Leboucq as reproduced by Broman ('11, i.e., fig. 460).

The other major deviation from the normal in this heart was the presence of a left superior cava, which received the blood from the superior intercostal and hemiazygos superior of the same side.

It may be noted in passing that this subject presented other minor aberrant conditions: an hiatus in the posterior arch of the atlas, a perforation of the sternum at the level of the fourth interspace, a supratorchlear foramen in each humerus, and a lateral internal mammary artery on the left side.

The interesting features in the specimen under discussion are as follows: 1) The presence of a left superior cava; 2) the right aortic arch with the associated relations of the left subelavian artery and ligamentum arteriosum, and, 3) the absence of the right common carotid.

The first point does not call for any discussion at this time. The condition is not especially rare and in this case the usual arrangement obtains in which there is no anastomosis between the two superior cavae (McCotter, '16).

The arterial arrangement is readily explained as may be seen in the diagram reproduced in figure 3. Right aortic arches are not particularly rare, Abbot ('92), Reid ('14), and many others have reported similar conditions. Poynter ('16) distinguishes four groups under the heading of 'right aortic arch.' Our case

represents the most common variety, in which there is an obliteration of the left fourth arch and of the dorsal roots beyond, with a persistence of the left dorsal aorta. The developmental history of these cases, as shown in figure 3, makes clear the low origin of the left subclavian from the descending aorta as well as

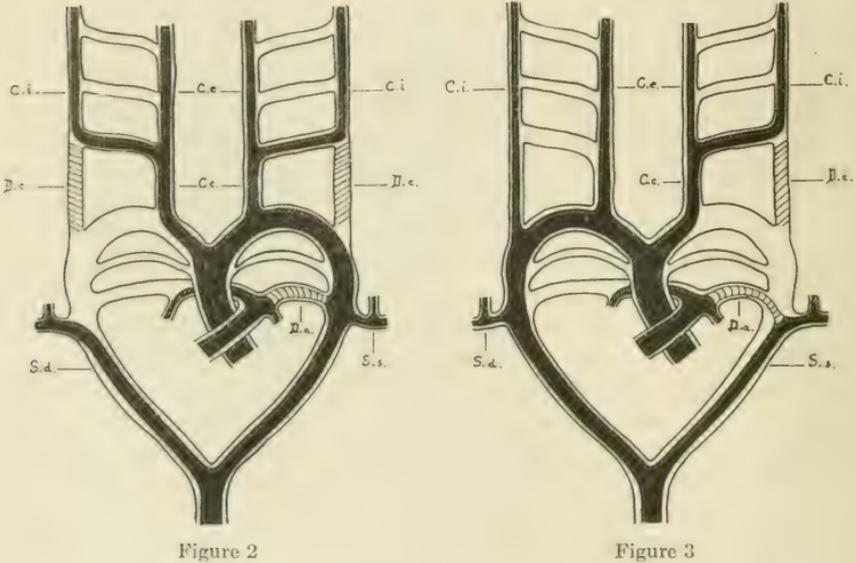


Figure 2

Figure 3

Fig. 2 Transformation of the aortic arches in those cases in which the right subclavian is the last branch of the arch.

Fig. 3 Present case, right arch with the usually associated type of left subclavian, absence of right common carotid.

C.c., carotis communis; *C.e.*, carotis externa; *C.i.*, carotis interna; *D.a.*, ductus arteriosus (ductus Botalli); *D.c.*, ductus caroticus; *S.d.*, subclavia dextra; *S.s.*, subclavia sinistra.

its relations to the ligamentum arteriosum. Almost constantly there is found a definite enlargement of the subclavian at its origin, and into this enlargement is inserted the remains of the ductus arteriosus. The ductus exhibits a remarkable constancy, it is rarely found on the right side even in cases of right arch.

At this point we would call attention to figure 2, which represents those relatively frequent cases in which the right subclavian is the last branch of the arch or arises from the descending aorta, and passes, in most cases, behind the oesophagus (cf. Holzapfel, '99; Banchi, '07). Associated with these atypical right subclavians, there is often a right thoracic duct. Except for the ductus Botalli, the more usual form of right aortic arch, and the right subclavian as the last branch of a left arch are mirror pictures of each other as may be seen from the accompanying diagrams. Both of these anomalous subclavians are in part dorsal aorta, but the commoner variety, on the right side, shows a much greater tendency to be shifted upward upon the aorta, closer to the left common carotid.

The carotids are of especial interest in the present case. As may be seen in the diagrams, the ventral aortae proximal to the fourth arches may give rise to a part of the aortic arch, a part of the common carotid or become the innominate. Although the carotids, the primitive and always the most important vascular channels to the head, may undergo extensive modifications and reductions, the absence of the common carotid constitutes one of the rarest arrangements of the branches of the aorta, and as an anomaly is very seldom encountered. Even a low bifurcation of the common carotid is extremely uncommon (Kantor, '05; Orr, '06). It is not necessary to explain the accompanying schematic diagrams, but we would direct attention to that portion of the dorsal aortic root between the third and fourth arches, shaded in the figures. This little stretch of dorsal aortic root is the ductus caroticus, sometimes erroneously referred to as the ductus Botalli. It is the persistence of this channel on the right side, with the loss of the third as well as the first two arches, which has given us the conditions present in this particular case.

Poynter in 1916 collected seven cases of absence of the common carotid, which is a good indication of the extreme rarity of the condition. The cases are briefly as follows: Malacarne's well-known case of double aortic arch with absence of the common carotids on both sides, 1784, figured by both Quain ('44) and Henle ('76); Power's case, noted by Quain, in which the right side was involved with apparently no other anomalies; Kantor (l.c.)

states that Kosinski ('76) described the separate carotids as arising from the innominate; Gottschau ('85), separate carotids on the left side, also other arterial variations; Macalister ('86) notes in a word the divided carotids on the right side; his second case, mentioned by Poynter, we have not been able to find; v. Angermayer ('06) described a simple case on the left side.

The rarity of the divided origin of the carotids is but an expression of the constancy with which the ductus caroticus becomes obliterated. This is one of the most constant features in the varying modifications undergone by the aortic-arch system in higher vertebrates. Remnants of all the arches—except possibly the fifth—as well as of the entire ventral and dorsal aortae persist in a variety of forms, but only very rarely does the ductus caroticus escape obliteration. This little bit of vessel does not seem to have received the attention which it deserves, its early and practically invariable disappearance is quite as significant as the equally invariable presence of the fourth arch. It is represented in almost all accounts of the aortic arches since the early representations of Rathke and Boas; sometimes miscalled, it is more often left without any designation whatever.

This interruption in the dorsal aortic roots, while not invariable, is yet well established in Reptilia. The results of this break are that the blood supply of the head becomes quite independent of that of the rest of the body. Further developments effect the remaining vessels, namely, the carotids, where extensive changes may occur. Instead of the four carotids, the blood supply of the head may be taken over by one carotid alone, and this either a ventral (ext.) or a dorsal (int.) vessel. These changes have usually been correlated with the elongation of the neck, birds and certain reptiles. A very primitive, symmetrical arrangement of the aortic arches, in which both ductus carotici and both ductus Botalli persist is found in that ancient and primitive form, *Sphenodon* (O'Donoghue, '17, '20).

In reporting this case it is not our purpose to enter upon any discussion regarding the possible factors involved, general configuration of neck or trunk, or the varying position of the heart and aortic arch (O'Donoghue, Parsons, '02), in determining either the definite vascular picture or the presence or absence of anomalies.

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Resumen por el autor, A. T. Rasmussen.

Sobre la organización de la neuro-anatomía para los estudiantes de medicina, basándose en una completa base funcional, en el caso de usar para la disección un solo cerebro humano.

El autor presenta en este trabajo un programa de trabajo diario durante un cuarto del curso el cual, según varios años de experiencia demuestran, ha dado satisfactorios resultados cuando se estudia el sistema nervioso humano mediante división en funciones. Las demostraciones semanales organizadas de un modo sistemático, en las cuales se presentan preparaciones normales, experimentales y patológicas de especial interés con referencia al punto que se está estudiando, son económicas y eficientes cuando cada preparación vá acompañada de notas explicativas suficientes. El autor menciona especialmente el valor de las preparaciones con el metodo de Marchi, especialmente en los tractos mezclados con otras fibras. También ilustra un esquema para resumir los sistemas funcionales importantes.

Translation by José F. Nonidez
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ON THE ORGANIZATION OF NEURO-ANATOMY FOR
MEDICAL STUDENTS UPON A THOROUGH-GOING
FUNCTIONAL BASIS WHERE ONLY THE HUMAN
BRAIN IS USED FOR DISSECTION

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SIX FIGURES

The report of the proceedings of the thirtieth annual meeting of the Association of American Medical Colleges shows that there still exists great diversity in the methods used in connection with the course in neuro-anatomy. The efficient teaching of this important and difficult course is undoubtedly one of the real problems before the medical schools. This is largely because of the complex nature of the subject and the limited time allowed for its study. The most economical utilization of the student's time is also becoming extremely important throughout the entire medical curriculum, due to the expansion and increased technicality of the subject-matter. On this account any suggestions for improvement which appear to develop as a result of the application of a particular method of handling a subject should be put on record that others may test it out. Too often a teacher is conducting a course as he was taught, irrespective of the efficiency of the method.

In connection with neuro-anatomy many valuable suggestions have appeared from time to time, such as the emphasizing of the functional aspect, methods of dissection which result in maximum exposure of internal structures, construction of large fiber-tract models, providing students with outline sketches to aid them in their work on the brain stem, etc. These all have their place and should be available and utilized. What is intended here is to outline the course as it is now given at the University of Minne-

sota, in order to show the practicability of proceeding upon a thoroughgoing functional basis. It is believed that the method of approach here used has a number of features which contribute to the economical expenditure of the student's time and ultimately to greater clearness in the physiological and clinical significance of the great array of anatomical details, which are too often left as merely a bewildering complexity.

This course on the gross and microscopic anatomy of the nervous system and organs of special sense is the outgrowth of many years of teaching and research by Dean J. B. Johnston (whose functional attitude and numerous publications in neurology are well known, in particular see "A new method of brain dissection," *Anat. Rec.*, vol. 2, p. 345, 1908) together with a number of minor modifications which seemed advisable to the writer after twelve years of experience with neurology in several universities.

Since only human brains are used for dissection, the approach to various structures must be carefully planned and plainly indicated to avoid undue destruction of things to be dissected later on in the course. It is not only possible, but practicable for each pair of students to get all the essentials upon one half of a human brain and have a respectable-looking dissection at the end of the course. It is, however, quite necessary to have available in the laboratory demonstration dissections and brains cut in gross serial sections in the vertical and in the horizontal plane. Such series will last for several years if the individual sections are about a centimeter thick and each series kept together in a jar sufficiently large to permit easy removal of the brain by the students. Brains from bodies in which the blood vessels have been injected with coloring matter are often especially good for this purpose, even if they have dried up to some extent. There is frequently an excellent differentiation of the white and the gray matter due to the gray having taken up more of the pigment from the injection mass.

The general plan of the course, after an introductory survey of the meninges, external anatomy of the brain and spinal cord and the methods utilized in working out the architecture of the nervous system, is to take up in turn each of the great afferent

conducting systems, commencing in each case with the receptors or end-organs, then reviewing the peripheral nerves and ganglia involved, and finally the fiber bundles and related gray matter in the spinal cord and brain to the highest known centers. As the tracts are followed upward in the central nervous system, the principal reflex connections encountered are indicated. After considering all the afferent systems except the olfactory—which is left till later in the course when the rhinencephalon can be most advantageously dissected—the cerebral hemispheres are taken up in the lectures under such headings as cause and significance of fissure formation, cerebral localization, histology of the cortex, association, commissural and projection fibers, while in the laboratory cerebral topography and histology of the cortex are considered and as much of the hemisphere dissected as is necessary to disclose the association, commissural and projection bundles. This prepares the students for taking up the efferent mechanism, although a general idea of the efferent limb of many reflex arcs has already been obtained incidentally in connection with the afferent system. The principal efferent systems are then traced to lower motor neurones whose relation to the peripheral nervous system and muscles and glands naturally follow. The olfactory system is then taken up. This leaves for final consideration the brain ventricles and the blood supply. Since during the course of dissection the blood vessels are not readily followed, demonstration specimens with vessels injected are used in the laboratory to elucidate the arterial supply and venous drainage.

Practically all anatomical structures are considered as they are encountered in following a conducting mechanism, and hence have a meaning which cannot be conveyed by merely stating that this is concerned with one function and that with another, which is about as far as many get along the line of functional analysis. Where the course is followed by a thorough review of the functional systems, the structures take on real meaning; but why not give the student this added interest while he is working out the structure? It is also believed that by this method there is obtained a better working knowledge of the inner architecture of the brain, because it is necessary to go up and down the brain

stem several times, each time following a particular conducting chain of neurones. The method also automatically eliminates from the course unnecessary details.

Each laboratory period is preceded by a lecture which prepares the student for the practical work to follow. After the completion of a certain phase of the subject, a series of about twenty-five demonstration preparations are shown, supplementing the lecture and accompanied by notes or a fully labeled sketch or both as the case may demand. Each laboratory section is assigned a particular hour for studying these preparations. If there are at least as many specimens as there are students in a section so that each student can be kept busy, a surprisingly large amount of information is obtained by the student in an hour.

While some say that pathological material is not necessary, a very effective means of stimulating interest and at the same time a method of presenting facts that cannot be seen in normal specimens is to demonstrate the results of known pathological or experimental lesions. Series of Marchi and Weigert preparations of degenerated tracts arranged in proper sequence showing the position and extent of the most important fasciculi through the brain stem and spinal cord are almost necessary to convince some students that the specific conducting bundles that are mentioned actually exist. Experimental and pathological material stained by the Marchi method is especially valuable at the beginning of the course to show how it is possible to follow a tract a long distance even when greatly mixed with other fibers. In demonstrating a long tract, the cross-sections of various levels should be arranged in proper order and the students made to go from microscope to microscope in the direction of nerve conduction. And certainly the significance of the architecture of the normal nervous system can be emphasized in no better way before medical students than incidentally to call attention at the appropriate time to such things as syringomyelia and disassociated sensibility; degeneration of the dorsal funiculi and ataxia; equilibratory disturbances, nystagmus, etc., in vestibular and cerebellar lesions; types of blindness resulting from lesions in different parts of the optic mechanism; differences in motor

disturbances in lesions at different levels of the pyramidal system, etc.

How the subject-matter can be arranged according to the above scheme to be given in three periods (total ten hours) per week during one quarter (twelve weeks) can best be seen from the following schedule. This assumes that it is not necessary to duplicate to any extent the embryology or general histology of nervous tissue, except in the form of demonstrations here and there in the course, which is especially the case where the neurology instructor handles the neural part of the regular courses in histology and embryology. We find it desirable to have the laboratory period on which the demonstration occurs three hours in length so as to leave two hours for regular laboratory work. On this period one laboratory section at a time is taken by the instructor into the demonstration room for one hour and then returned to the regular laboratory to allow the next section to come to the demonstration.

It is, of course, not practicable to have the lectures and laboratory work run exactly parallel throughout, since the time that can profitably be spent in lectures does not vary from subject to subject directly with the time necessary to cover the practical side. Thus the first time a long conducting system is followed through the brain stem, sufficient time must be allowed to get some of the prominent land-marks and make a limited number of outline sketches illustrating typical levels. Structures not directly involved in the system followed need not be sketched in detail nor at all if not readily recognized for the time being. Like the method advocated by Doctor Lineback for embryology and used by Doctor Herriek in neurology, details are added to these drawings as subsequent systems are considered. If the particular slides to be outlined are carefully selected for the student, seven sketches will usually suffice to show all the essential internal structures of medulla oblongata, pons, midbrain, and diencephalon, including the nuclei and root fibers of the cranial nerves. For convenience these seven levels above the spinal cord are numbered from 1 to 7, from below upwards. Each student's loan collection contains many intermediate levels (from 24 to 50), which he is expected to

study. All the structures belonging primarily to a given system may be indicated, as is frequently done, by the use of a particular color.

The total number of sketches of microscopical preparations and of dissections required is twenty-eight. Copies of several charts are also called for. Naturally these must be made as simple as possible, for time does not permit the making of what some would call presentable drawings, and yet, as most agree, some sort of record seems desirable. There is, of course, no time for making microscopical preparations which students may claim as their own. Those who agree with Doctor Wells that students should come to pathology with a personal collection of normal preparations will have to show where more time is available for microscopic anatomy, since it is frequently a question of utilizing the available time in either making slides or in studying them. The statement that the loan collection is merely an easy way of getting around difficulties is unjustified in most cases, if not in all.

Two preliminary written examinations of one hour each are given during the course--one in the fourth week and another in the eighth week--sometimes in the lecture period and other times during a laboratory period, depending upon where the time can best be spared. Several short practical quizzes are given at irregular intervals. The final test consists of a two-hour written and a one-hour practical examination.

Quarterly schedule

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
1	Meninges and meningeal spaces Gross Microscopic	Meninges, gross and microscopic Dem. 1—Meninges. Cytology of nerve cells, nerve fibers, and neuroglia
2	Neurological methods General morphological Methods involving nerve degeneration	External features of spinal cord and rhombencephalon (omitting, temporarily, details on cerebellar lobes and fissures)
3	Special histological (Cajal, Golgi, etc.) Physiological Pharmacological	External anatomy of mesencephalon and diencephalon Dem. 2—Degeneration and regeneration of nerve fibers
4	Pain, touch, and temperature system commenced Tactile corpuscles	External topography of the telencephalon
5	Peripheral nerves and ganglia involved and their structure	Spinal gang., microscopic structure Begin microscopic structure of the spinal cord
6	Spinal cord Microscopic structure Reflex possibilities	Spinal cord, microscopic structure Dem. 3—Sensory nerve endings, spinal ganglia, spinal cord
7	Conduction in spinal cord of pain, touch, temperature Spinal V. tract and its relation to Nn. V., IX., X.	Lower medulla oblongata (level 1) Microscopic structure with special reference to spinal lemniscus, spinal V. tract and its nucleus
8	Spinal and trigeminal lemnisci to the thalamus Internal capsule and Cortical areas related to pain, touch, temperature	Upper medulla oblongata (level 2) and Lower pons (level 3) Microscopic structure, special reference to pain, touch, temperature conducting system
9	Deep sensibilities (muscle sense, etc.) system commenced Muscle and tendon spindles Peripheral nerves involved	Pons at entrance of N. V. (level 4) Lower midbrain (level 5) Microscopic structure with special reference to pain, touch, etc.
10	Course within spinal cord Dorsal funiculi Spinocerebellar tracts	Upper midbrain level (6) Microscopic, special reference to spinal and trigeminal lemnisci

Quarterly schedule—Continued

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
11	Cerebellum Gross structure Localization of function Microscopic structure Components of peduncles	Diencephalon (level 7), gross and microscopic series. Special reference to spinal and trigeminal lemnisci, lateral nucleus of thalamus, internal capsule
12	Brachium conjunctivum Medial lemniscus Lateral nucleus of thalamus Cortical areas related to deep sensibility system	Review brain-stem series, noting and inserting on sketches the structures of deep sensibility Dem. 4—Marchi and special slides on tracts of deep sensibility
13	Vestibular (equilibratory) system Inner ear, general structure Vestibular part in detail	Cerebellum. Review gross structure Dissection of peduncles Microscopic structure
14	Vestibular nerve and nuclei Vestibular tracts in the brain-stem, spinal cord, and to the cerebellum	Vestibular structures followed in brain-stem series and inserted in sketches of the proper levels already outlined
15	Auditory system Ear, microscopic structure of external and middle ear, cochlea	Cochlea, microscopic structure Dem. 5—Special slides on cerebellum and ear. Models of ear. Marchi series on vestibulospinal fasciculus
16	Cochlear nerve and nuclei Lateral lemniscus Cortical connections Reflex possibilities	Tracts and nuclei of auditory system followed in brain-stem series and inserted on outlines of proper levels already made
17	Visual system Eyeball Gross structure Microscopic structure	Eyeball Dissection of beef eye Microscopic structure, excluding the retina
18	Retina in detail	Retina and optic nerve, microscopic Dem. 6—Models and special slides on the eye and optic nerve
19	Optic nerve and chiasm Optic tract and connections Visual cortex Reflex connections	Study gross and microscopic material on upper midbrain and metathalamus and insert on sketch of level 6

Quarterly schedule—Continued

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
20	General visceral afferent system End organs, peripheral nerves Pathways in cord and brain Gustatory system Taste buds Peripheral nerves involved Central connections	Review brain-stem series for Nn. VII., IX. and X, and Solitary fasciculus and nucleus and insert on sketches Dissection of solitary fasciculus Taste buds Microscopic structure
21	Cerebral hemispheres Weight with age, sex, etc. Significance of fissures	Study gross divisions of cerebral hemispheres on brains being dissected and on gross series
22	Cerebral localization	Review cerebral topography
23	Histology of cerebral cortex	Histology of cerebral cortex
24	Association, commissural and projection fibers	Histology of cerebral cortex Dem. 7—Histology of cerebral cortex
25	Somatic motor system Motor cortex Pyramidal system	Dissection of association bundles, lenticular nucleus, internal capsule pyramidal fibers
26	Cortico-pontile system Extrapyramidal system Corpus striatum	In gross brain series study form and relations of internal capsule and corpus striatum
27	Lower motor neurones Cranial nerves Spinal nerves Variations and double innervation in muscles Motor end plates	Study somatic motor structures in brain-stem series and spinal cord Dem. 8—Histology of corpus striatum and subthalamic region. Marchi series on pyramidal and rubrospinal tracts. Motor end plates
28	Special visceromotor system Visceral striated muscles General visceral efferent system Sympathetic ganglia	Study brain-stem series on visceral effective nuclei and nerves Complete brain-stem sketches Histology of sympathetic ganglia
29	Craniosacral division (autonomic, parasympathetic) Relation to prevertebral and terminal ganglia	Copy charts on craniosacral outflow showing course of preganglionic and postganglionic fibers to particular viscera

Quarterly schedule—Concluded

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
30	Thoracolumbar division (sympathetic proper) Special relation to sympathetic trunk and vertebral ganglia	Copy chart on thoracolumbar division Dem. 9—Special slides and dissections on sympathetic ganglia, nerves, rami communicantes
31	Olfactory system Olfactory membrane, nerves, bulb, tract, central connections	Histology of olfactory membrane, and olfactory bulb Dissection of rhinencephalon
32	Ventricles of the brain Topography, significance Ependyma, chorioid plexus Cerebrospinal fluid	Dissection of brain completed Topography of the ventricles Chorioid plexus Caudate nucleus
33	Arterial supply and venous drainage of the brain	Blood supply of the brain and cord Dem. 10—Special preparations on olfactory system, ependyma, chorioid plexus and ventricles

SUMMARIZING SCHEMES

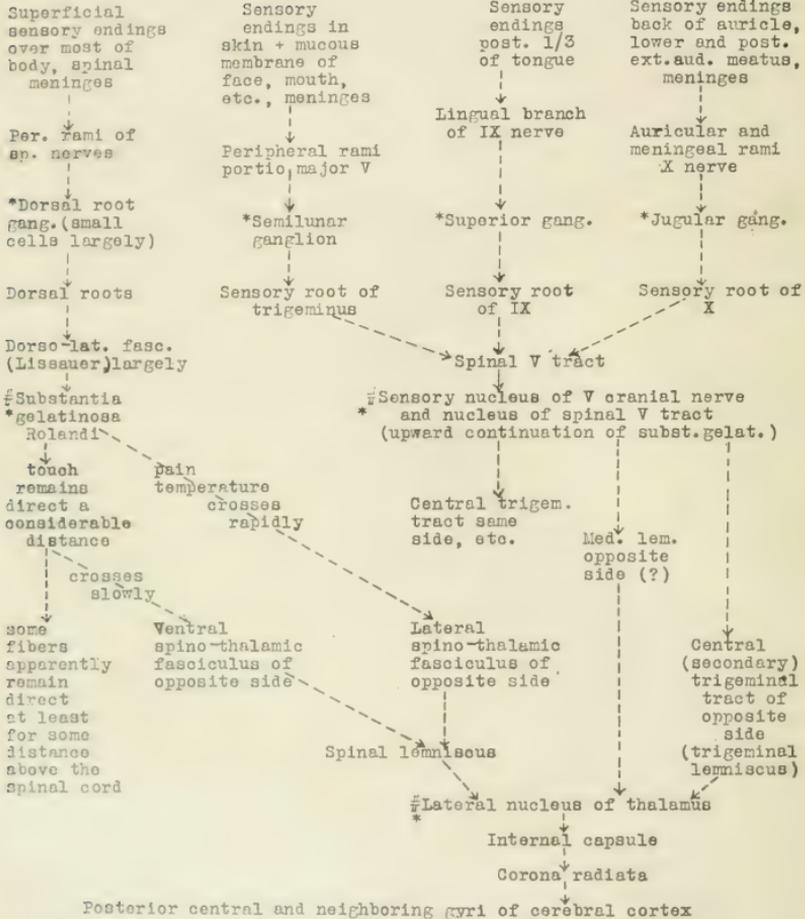
In addition to large charts and models showing the position and relations of the structures by functional systems, schemes such as are shown below have been received with sufficient enthusiasm by our students to suggest that others might find them useful. They are necessarily too dogmatic and must be interpreted liberally in places where uncertainty still exists. Such points are, of course, covered in the lectures.

The general idea underlying the arrangement has been to make a compact and suggestive summary of each functional system. In many cases there has been sufficient space to put coordinate structures in about the same plane. The items have been so spaced and abbreviated that each system can be type-written on an ordinary-sized stencil (size $8\frac{1}{2}$ inches x 11 inches). All lines may be put in by hand or the vertical ones may be made by machine. The arrow-heads are stenciled in with a stylus shaped like a small sharp screwdriver.

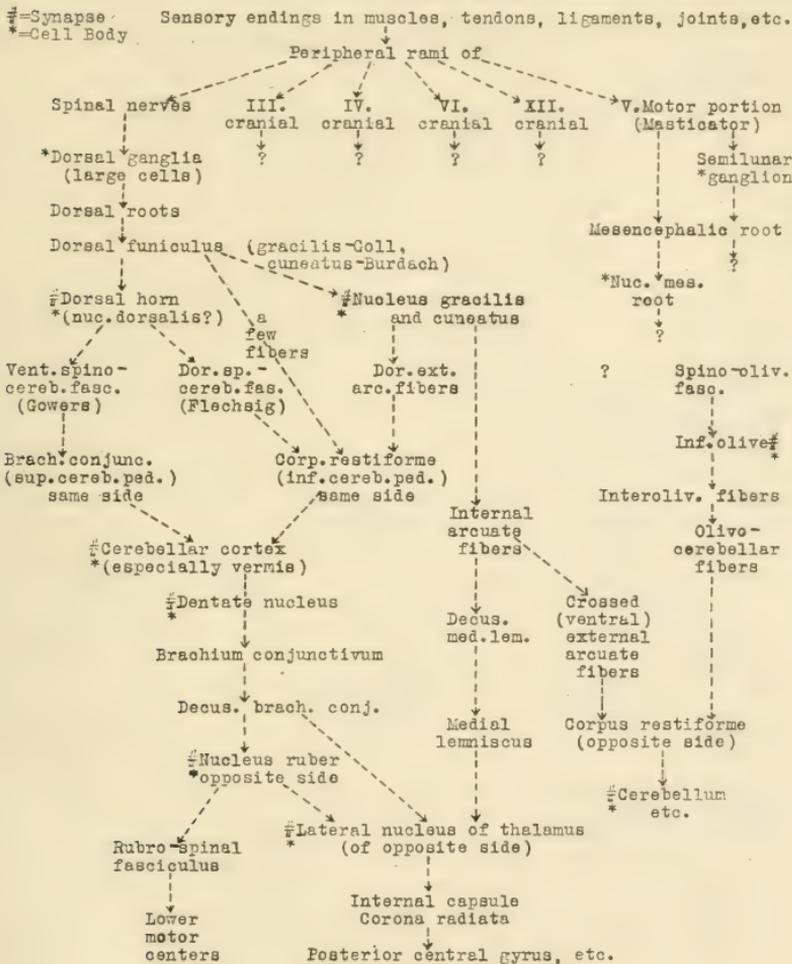
It may be objected that such tabulations will be used as short-cuts and memorized at the expense of a clear idea of the position and relations within the nervous system. Students should be cautioned about this and required to pass practical examinations. On the other hand, it is a method of organizing numerous details into important units in such a fashion that it is a distinct aid to many students.

PAIN - TOUCH - TEMPERATURE

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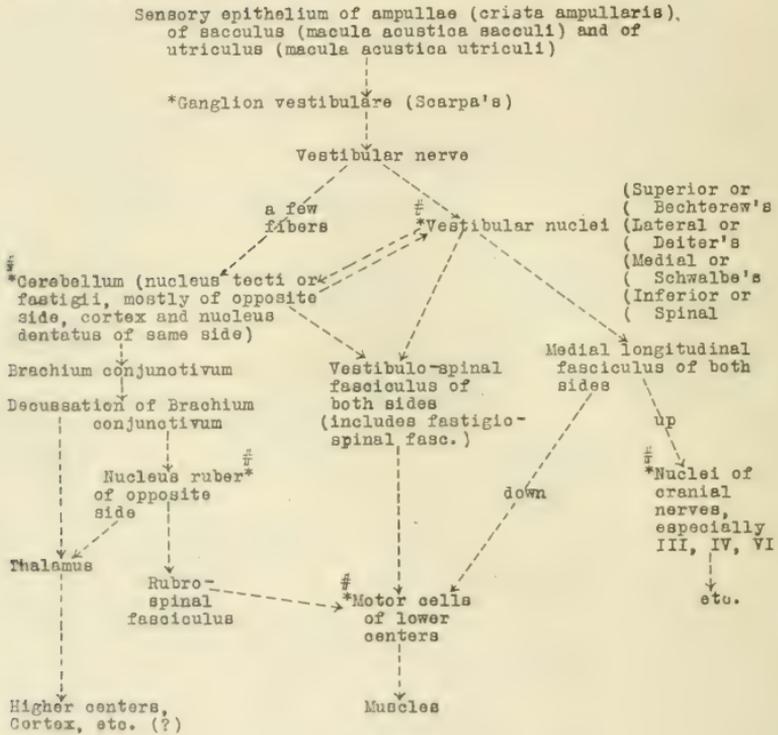


DEEP SENSIBILITY (Muscle Sense, Vibratory Sense, etc., Sense of Position of Members of the Body and of Passive Movement) -
 SPATIAL DISCRIMINATION (Two-point; Size, Shape and Texture of Surfaces; Stereognosis)



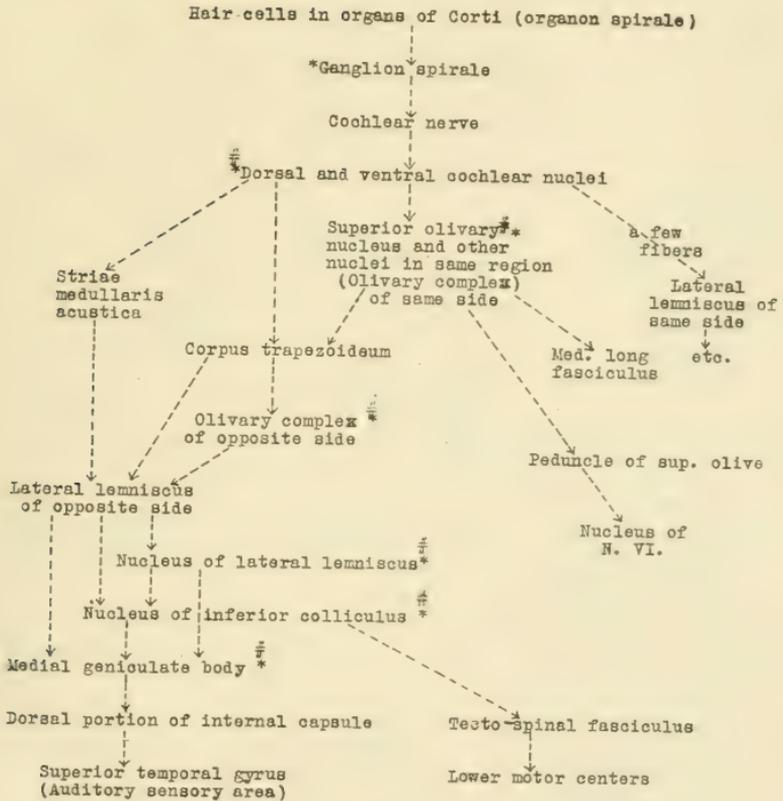
VESTIBULAR OR EQUILIBRATORY SYSTEM

‡=Synapse
 * =Cell body



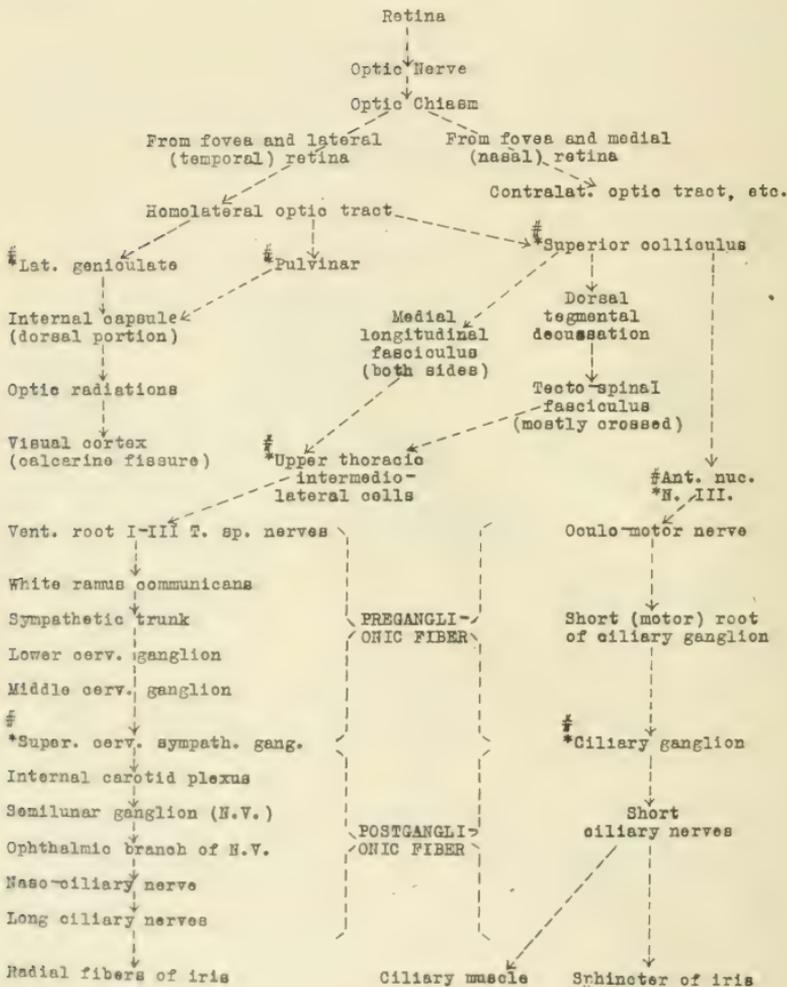
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AUDITORY SYSTEM



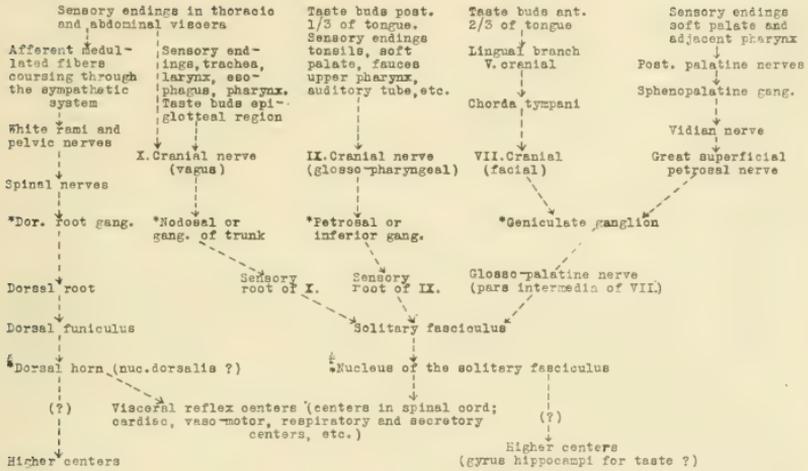
*=Cell Body.
 ‡=Synapse.

VISUAL SYSTEM



GENERAL VISCERAL RECEPTIVE AND GUSTATORY SYSTEM.

‡=Synapse
 *—Cell body



Resumen por el autor, Stacy R. Guild.

Un método de reconstrucción gráfica para el estudio del órgano de Corti.

La mayor parte de los investigadores han descrito las lesiones cocleares en documentos escritos acompañados de diagramas de secciones midmodiolares, estimando de modo aproximado la longitud relativa de las diversas lesiones, generalmente en términos de las partes aproximadas de las vueltas de espira, sin haber evaluado la longitud de dichas espiras. Una interpretación mejor de los resultados puede conseguirse por el método previo del autor, que consiste en representar el órgano de Corti como una banda derecha con las longitudes relativas de las medias vueltas de espira aproximadas; pero puesto que el valor linear de las diversas partes de una estructura curva contenidas en secciones radiales, oblicuas y tangenciales varía, la localización de los extremos de las lesiones solo puede ser aproximada. El método de representación gráfica que el autor describe en el presente trabajo, evalúa directamente las longitudes aproximadas de todas las porciones del órgano de Corti en los cortes seriados. En papel rayado cada espacio representa un corte; se determinan los cortes limitantes de cada media vuelta de espira y se dibuja una serie de semicírculos uniendo los puntos así hallados. El resultado es una curva semejante a una espiral que permite aproximadamente las variaciones de los valores lineares de diferentes secciones. Esta es la línea basal para representar gráficamente los datos que se desee. Los tantos por ciento de error para las diferentes porciones, determinados para seis gráficas, van incluidos en el trabajo. Los datos más exactos obtenidos por este nuevo método pueden transportarse a gráficas rectas para facilitar la comparación de numerosas observaciones. Este método no solo susministra datos más exactos sino también (en el caso de ser adoptado por otros investigadores en este campo), permite poner en posesión de otros los resultados en una forma que facilita una comparación más exacta que hasta el presente ha sido posible.

A GRAPHIC RECONSTRUCTION METHOD FOR THE STUDY OF THE ORGAN OF CORTI

STACY R. GUILD

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ONE CHART AND THREE FIGURES

Many papers recording the lesions found in the cochleæ of animals submitted to experimental injury by various sounds, both pure tones and detonations, have appeared in the last fifteen years. For the purposes of this communication it is unnecessary to review these, other than to mention a few of the authors. There have been several papers by Wittmaack ('07, '09, '18) and his co-workers; a series of very good works have appeared from Siebenmann's laboratory at Basel, those by Yoshii ('09), Hoessli ('12, '13), and Satoh ('17, on birds) being prominent; a long report by Hoshino ('17) on detonation injuries and more recently one by Guild ('19) on detonation injuries of exposed and protected ears. The above are only a part of the experimental sound-injury records which have appeared.

The value of these studies in the ultimate solution of the problem of the physiology of hearing is in direct ratio to the accuracy with which the position and extent of the observed lesions have been recorded; provided, of course, that the histologic technique is satisfactory. Most workers in this field have recorded their observations by individual written protocols for each animal, describing by text matter or drawings the nature of the lesions and indicating their extent and position by such very general terms as "upper part of the basal turn," "almost all of the second turn," or "lower half of third turn and upper part of second turn." Such records are, at best, very indefinite, as they make a comparison of extents of injured areas almost impossible, as will become evident later. No attempt is made in such reports to indicate the

difference in length of the various turns, and it will appear later in this paper that only the very roughest of estimates could be made thus of the relative linear values of the different lesions. They do, of course, serve to indicate in a general way the relative positions of the various lesions in the cochleæ, and have been of great value thus. The only attempts at graphic summaries of the results have been diagrams of midmodiolar sections with the tone designation or the name of the gun (Hoshino) affecting the general region indicated in the various levels of the cochlear duct shown in the section (fig. 1).

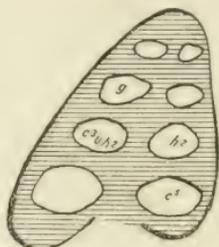


Fig. 1 Copy of a text figure in article by Yoshii ('09). This is an illustration of the method referred to in the text of diagrammatic localization of lesions with midmodiolar sections. It has been employed in connection with individual protocols and gives but a very rough idea of relative extents and positions of lesions.

While working on the problem of war deafness and the effectiveness of protective measures against detonation injuries, the writer recognized the need for a more accurate recording of the position and extent of the lesions and the desirability of having the data in a form which renders possible a more exact comparison of one record with another. While the method presented in the present communication was worked out then in its general features it was only partly made use of because of the need for haste at that time and the fact that the determination of the relative efficiencies of various forms of protection of the ears does not require the degree of accuracy in the location of the lesions that the work on the physiology of hearing requires. The relative lengths of

the various half-turns of the organ of Corti of the animals used (guinea-pigs) were approximated by actual measurements of one cochlea, and the organ of Corti represented graphically by parallel spaces in charts, with the various half-turns marked off by vertical rulings. By shades of gray and other symbols, the nature of the lesions in the various parts of the different cochleæ was shown, but the positions of the ends of the areas injured were only approximations, although serial sections were used. The reason for this is self-evident when one recalls the continuous change in linear value of each section of the organ of Corti as it

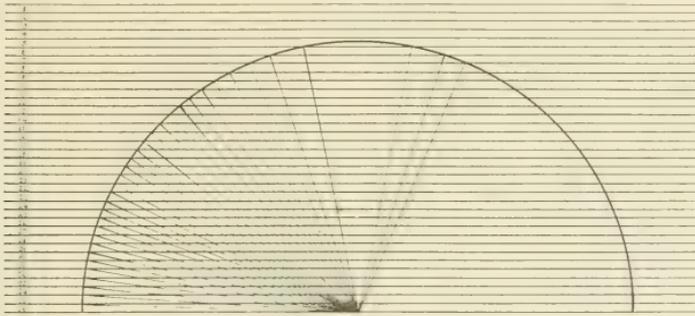


Fig. 2 Diagram to illustrate the changing linear value of the parts of a curved structure such as the organ of Corti included in sections of the same thickness. This diagram shows at a glance why the simple counting of the number of sections through which lesions pass gives but a very rough idea of the relative lengths.

passes from 'radial' to 'tangential' sections (fig. 2). A simple count of the number of sections does not give the relative lengths of any two parts of the given organ of Corti. But the method of recording used in that report (Guild, '19) permitted of a much better comparison of the lesions in various cochleæ than would previous methods not only by the author, but by any one else interested. (Chart 1 is reproduced herefrom the author's previous publication to illustrate the method.)

The method of determination of the position and extent of lesions of the organ of Corti which will now be presented is one which the author believes will improve the accuracy of the state-

Series	1 st Half Turn	2 nd Half Turn	3 rd Half Turn	4 th Half Turn	5 th Half Turn	6 th Half Turn	7 th Half Turn	8 th Half Turn
1								
2								
3								
4								
5								
6								
7								
8								
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10								
11								
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100								

Chart 1 Copy of a chart in article by Guild (19). In this method of recording the relative lengths of the several turns have been approximated, so that better estimates could be made of the lesions. Such a chart as this may also be employed in order to group and thus facilitate comparison of the more accurate data obtained for each cochlea by the graphic method described in this paper. (The various symbols and shades of gray indicate types of lesions; for the interpretation of these reference should be made to the original paper.)

ments of location and relative extents of the injuries from various causes and which will, if adopted by other workers in this field, render the results of all available to the others in a form which will permit of ready comparison. This would, without question, be desirable and would facilitate the solution of the problem of the physiology of hearing.

METHOD OF THE GRAPHIC RECONSTRUCTION

Prerequisites to the use of the method are, 1) good fixation and embedding of the cochleæ, and, 2) serial sections of these cochleæ in the approximate plane of the modiolar axis. This is the plane in which cochleæ are most often cut for study. Loss of sections or lack of uniformity in thickness of the sections interferes with the use of this method, just as such imperfections render any reconstruction method unsatisfactory. The method most used by the author in preparing cochlear material has been fully described elsewhere (Guild '19). In brief, it consists of injection fixation with warm Zenker formalin solution followed by double embedding in celloidin and paraffin: most series have been cut at $7\ \mu$; this, being about the thickness of the hair cells of the guinea-pig cochleæ, gives preparations which are better for most purposes than either thicker or thinner ones.

On closely ruled paper, such as that known as 'profile paper,' which has 400 spaces in a 20 inch width, each space is considered as representing a section, and a preliminary to starting a given reconstruction is the assigning of definite designations to as many spaces as there are sections involved in the part of the series to be plotted. The author has done this by actually writing the slide, row, and section number (in the row) in a column at the left end of the paper, rather than by designation by serial numbers of the total series of sections. This makes it very easy to identify corresponding sections and spaces at any time. With the guinea-pig cochleæ cut at $7\ \mu$, the basal turn of the organ of Corti (not the bony wall of the cochlea) usually extends over something more than 300 sections. As a 'base-line' the center of the union of the outer and inner pillar cells has been selected. The exact sections in which this union of the pillar cells is cut tangentially are deter-

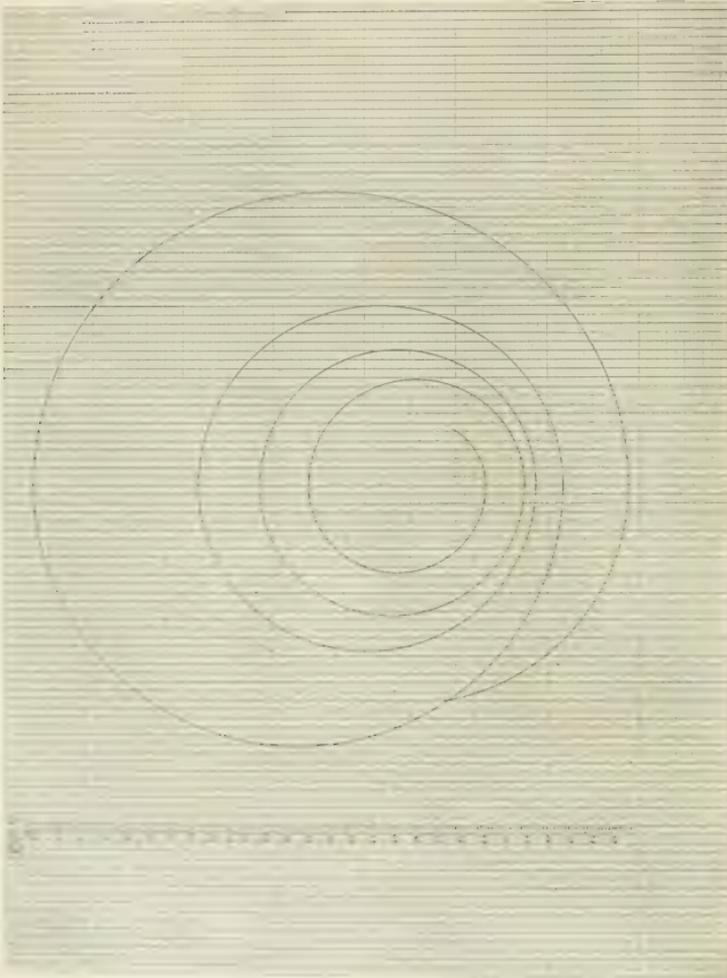


Fig. 3. Graphic reconstruction of the line of union of the outer and inner pillar cells of the organ of Corti in a given cochlea (guinea-pig no. 66 Rt.). (Reproduced at $\frac{1}{3}$ actual size of graph). This line serves as the base line for charting any desired data of the organ of Corti. Secondary concentric lines may be drawn at the correct distances to indicate rows of hair cells. Each space of the ruled paper represents a section of the cochlea, and definite designations are given the spaces. The paper used has 400 spaces in a width of 20 inches. For the method of drawing graph see text matter, page 145.

mined and indicated by marks in the correct spaces along a central line at right angles to the spaces representing sections. The tangential cuts of the organ of Corti are designated as the junction regions of the various 'half-turns' into which the cochlea may be considered as divided for descriptive purposes. Then the various marks representing the graphic positions of the half-turn junctions are connected by semicircles, each semicircle representing a half-turn of the cochlea; the centers of the semicircles are all on the common central line and the curvatures of successive circular arcs are such that there is no abrupt break evident to the eye. The graphic representation of the fractional turns beyond the last tangential cuts of the organ of Corti must be determined by a somewhat different method, since they end away from the central line. For each end the procedure is the same: the section in which the last union of pillar cells occurs is found and the distance measured by the usual microscopic methods between this union and the union of pillar cells of the full half-turn continuous with it (being first half-turn for the basal end and eighth half-turn for the apical end in the guinea-pig). This distance is multiplied by the scale of the graph and then laid off along the space of the graph which represents the given section, one end being at the place where the line of the full half-turn concerned crosses the space, and the other end on the opposite side of the central line. This determines the position of the end of the fractional turn on the graph; the other end of this fractional turn is of course where the adjacent half-turn joins it at the central line. A circle is now determined which has its center on the central line and a radius such that the circumference passes through both of the above points, and an arc drawn ending at these points. The fractional turn at the basal end has been designated the 'vestibular part' and that at the apical end the 'apical part.'

The resulting 'base-line' is a continuous curved line somewhat like a spiral in general appearance, although far from it mathematically (fig. 3). The author fully realizes the sources of error introduced: among these are: 1) the change in curvature in the cochlea is probably continuous rather than abrupt; 2) the half-turns are not true semicircles, and 3) the fact that no allowance

has been made for the 'basi-apical' distance traversed in the different parts. However, the author believes that a more exact approximation to a true graphic representation of length of the organ of Corti would involve so much mathematical calculation in the three dimensional determinations of points and their projections onto a plane surface that but few, if any, otological workers would have either time or facilities to carry out the procedure; whereas, the method here described requires only the use of microscope, drawing-board, ruled paper, and compasses, and is quickly done.

With such a base-line established, similar 'concentric' secondary lines may be drawn at the graphic distances of the various rows of hair cells, and the spaces between these concentric lines regarded as occupied by the hair cells. Then, in whatever order the serial sections are studied, the condition of the organ of Corti in each turn of each section, even to the individual hair cells, may be recorded by any suitable notation of colors or hatching.¹ By this method the position and extent of the various lesions present are determined to a degree of accuracy hitherto not attained in the recording of such lesions, and to a degree which will permit much better correlation of the work by all interested in the problem of the physiology of hearing.

CALCULATION OF ERRORS

The writer has determined the approximate error of this method of reconstruction of the organ of Corti by the following means. The magnification at which any graph is made is determined by the ratio of the thickness of the sections of the series to the width of the spaces of the ruled paper. In any section in which two places of cutting of the union of the pillar cells are less than a half-turn apart the actual distance may be measured by the usual microscopic methods and compared with the distance on the graph between the places where the line representing the union of the

¹ In order to avoid the confusion of the hatching lines by the rulings of the paper, the author plans to use tracing paper over the profile paper; this will make it much easier to disregard the section lines of the paper and yet permits them to be seen at any time for the determination of location.

pillar cells crosses the space representing the given section. This is of course a comparison of the chords subtending the arcs of the organ of Corti and of the graph rather than a comparison of the arcs themselves, but it seems reasonable to assume that the ratios of arcs and chords of these but slightly differing curves are such that the error introduced here is well within the limits of accuracy of the method in other respects. In order to determine the percentage value of the errors, the actual distance along a section was multiplied by the scale of the graph and this figure used as the denominator of a fraction of which the actual length along the corresponding space on the graph is the numerator. Evaluation of this fraction and the proper conversions give the result in the usual percentage terminology.

In order to determine the probable errors inherent in the method the writer has measured, by the procedures above described, the chords subtending the arcs of the base-line in either each tenth or each fifteenth section in each turn of each of six reconstructions. In table 1 are shown the detailed figures for one series; and in table 2 are shown only the figures giving percentage of error found by each measurement in the six series. In these tabulations, in the listing of the figures for each turn, the order of the sections measured is always the same; the order being from the tangential toward the radial or modiolar sections. This arrangement of the data calls attention to an interesting fact: i.e., the change in percentage of error is usually a continuous one and the direction of this change in value, when arranged with tangential sections first, is usually from positive numbers toward negative numbers (plus toward minus) whether the zero point is crossed or not. So constant is this that after it was observed the finding of a number out of order raised a question of error in computation and a complete recalculation was made. As a result those that are out of this order in the tables have all been rechecked until the writer is certain that they are not errors in computations. It is entirely possible that this progressive change in the percentage of error in each turn is due in some way to the semicircle combinations used in plotting.

TABLE I

Figures on which are based the percentages of error for the parts of the graph of the right cochlea of guinea-pig no. 66 (see text matter, p. 148, for explanation)

MEASURED FROM	SLIDE, ROW, SECTION	ACTUAL DISTANCE	ACTUAL DISTANCE MULTIPLIED BY SCALE OF GRAPH	DISTANCE ON GRAPH ALONG LINE OF SECTION	PERCENT- AGE OF ERROR IN GRAPH
		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
Vestibular part to 1st half- turn	6-2-11	0.713	129.4	129.5	+0.05
	6-2-1	0.969	175.9	182.5	+3.77
	6-1-3	1.177	213.6	220.7	+3.35
	5-4-12	1.429	259.3	262.8	+1.35
	5-3-9	1.598	289.9	294.5	+1.57
	5-2-6	1.729	313.6	318.4	+1.29
	5-1-3	1.831	332.3	335.8	+1.06
	4-5-5	1.902	345.1	345.1	0.00
1st half-turn to 2nd half-turn	1-4-9	0.704	127.7	125.0	-2.13
	1-5-6	1.075	195.1	184.5	-5.43
	1-6-3	1.324	240.1	225.8	-5.97
	1-7-5	1.601	290.5	272.5	-6.19
	2-1-8	1.807	327.8	307.5	-6.21
	2-2-11	1.967	356.9	334.5	-6.26
	2-4-2	2.093	379.7	355.2	-6.46
	2-5-6	2.206	400.3	371.3	-7.24
	3-1-3	2.294	416.1	382.8	-8.01
	3-2-6	2.357	427.6	390.0	-8.78
2nd half-turn to 3rd half-turn	5-4-12	0.602	109.2	117.6	+7.73
	5-4-2	0.863	156.6	166.8	+6.49
	5-3-1	1.047	189.9	200.7	+5.71
	5-2-1	1.256	227.9	238.5	+4.66
	4-5-10	1.402	254.4	265.3	+4.30
	4-4-7	1.508	273.6	284.8	+4.08
	4-3-4	1.581	286.8	298.0	+3.91
	4-2-1	1.624	294.6	305.4	+3.66
3rd half-turn to 4th half-turn	2-5-1	0.449	81.4	84.5	+3.78
	2-5-11	0.727	132.0	134.8	+2.16
	3-1-3	0.907	164.5	167.3	+1.72
	3-2-6	1.100	199.6	200.7	+0.55
	3-3-9	1.232	223.5	222.5	-0.45
	3-4-12	1.312	238.0	236.3	-0.70
	4-1-3	1.353	245.4	243.5	-0.79

TABLE 1—Continued

MEASURED FROM	SLIDE, ROW, SECTION	ACTUAL DISTANCE	ACTUAL DISTANCE MULTIPLIED BY SCALE OF GRAPH	DISTANCE ON GRAPH ALONG LINE OF SECTION	PERCENT- AGE OF ERROR IN GRAPH
		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
4th half-turn to 5th half-turn	5-3-9	0.500	90.7	95.2	+4.96
	5-2-11	0.727	131.8	134.8	+2.23
	5-2-1	0.884	160.3	161.1	+0.48
	4-5-10	1.039	188.4	187.7	-0.38
	4-4-7	1.131	205.2	203.8	-0.67
	4-3-4	1.199	217.5	211.8	-2.63
5th half-turn to 6th half-turn	3-2-6	0.437	79.4	81.0	+2.08
	3-3-4	0.662	120.1	120.8	+0.64
	3-4-2	0.809	146.7	146.0	-0.49
	3-5-5	0.954	173.1	170.2	-1.68
	4-1-8	1.039	188.5	183.6	-2.61
	4-2-11	1.086	197.0	188.5	-4.31
6th half-turn to 7th half-turn	5-3-4	0.438	79.5	78.9	-0.79
	5-2-6	0.648	117.5	114.4	-2.67
	5-1-8	0.779	141.4	136.5	-3.47
	4-5-5	0.909	165.0	156.3	-5.28
	4-4-2	0.972	176.4	165.5	-6.16
7th half-turn to 8th half-turn	3-4-7	0.337	61.2	59.3	-3.18
	3-5-5	0.545	98.9	95.5	-3.43
	4-1-3	0.669	121.3	116.1	-4.31
	4-2-1	0.743	134.8	128.2	-4.92
	4-2-11	0.785	142.5	134.8	-5.40
8th half-turn to apical part	5-1-8	0.302	54.9	58.6	+6.83
	5-1-3	0.400	72.5	77.0	+6.20
	4-5-9	0.484	87.8	87.8	0.00

In studying table 2 it is observed that the general nature of the differences between the measurements of the actual cochlear sections and of the graph for the corresponding turns of the several cochleæ is the same. Thus, for the measurements between parts of the first and second half-turns the figures giving the percentage of error are in each case negative, and are larger in the modiolar region than any other figures for the same cochlea. This simply means that the true curvature of the basal turn differs

TABLE 2

Percentages of error as determined for each of six cochleae (see text matter, p. 149, and table 1 for explanation)

		ANIMAL NUMBER AND SIDE						
		64 Left	65 Right	66 Right	67 Right	68 Right	69 Right	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Vestibular part to 1st half-turn		+4.47	-1.20	+0.05	+ 4.25	- 1.36	+13.28	
		+1.62	-1.22	+3.77	+ 5.13	- 0.05	+ 9.47	
		+0.67	-2.18	+3.35	+ 4.16	+ 0.97	+ 7.56	
		+0.55	-0.95	+1.35	+ 3.61	+ 1.49	+ 6.17	
		+0.81	-0.88	+1.57	+ 2.73	+ 1.75	+ 3.38	
		+0.50	-0.00	+1.29	+ 1.49	+ 1.47	+ 2.37	
		0.00	-0.07	+1.06	0.00	+ 1.24	0.00	
			0.00	0.00		0.00		
			-4.62	-2.40	-2.13	- 8.01	-10.76	-10.28
			-5.13	-3.77	-5.43	- 9.38	-10.21	-11.14
1st half-turn to 2nd half- turn		-6.29	-5.08	-5.97	- 9.17	- 9.78	-11.62	
		-6.47	-5.04	-6.19	- 9.74	- 9.71	-11.55	
		-6.99	-5.36	-6.21	-10.32	- 9.03	-11.65	
		-7.05	-5.52	-6.26	-10.71	- 9.18	-12.28	
		-7.76	-5.58	-6.46	-11.49	- 9.70	-13.20	
		-8.39	-6.80	-7.24	-11.87	-10.41	-14.08	
		-8.62	-7.44	-8.01	-13.12	-10.87		
		-9.32		-8.78				
			+6.23	+6.92	+7.73	+ 7.85	+ 1.90	+ 4.87
			+5.54	+4.08	+6.49	+ 5.75	+ 2.05	+ 4.57
2nd half-turn to 3rd half- turn		+4.38	+4.56	+5.71	+ 3.98	+ 2.02	+ 2.64	
		+4.01	+4.28	+4.66	+ 3.73	+ 1.92	+ 2.39	
		+3.65	+3.18	+4.30	+ 3.29	+ 1.58	+ 2.27	
		+3.32	+2.65	+4.08	+ 2.75	+ 1.49	+ 2.21	
		+3.00	+2.94	+3.91	+ 2.47	+ 1.36	+ 1.33	
		+2.33	+2.81	+3.66			+ 1.17	
			+1.86	+4.39	+3.78	- 0.62	+ 4.42	- 3.08
			+0.17	+3.02	+2.16	- 0.88	+ 2.06	- 1.81
3rd half-turn to 4th half- turn		-0.57	+1.51	+1.72	- 0.58	+ 0.11	- 1.36	
		-0.88	+1.34	+0.55	- 1.26	- 0.34	- 2.08	
		-1.31	-0.30	-0.45	- 1.86	- 0.73	- 2.41	
		-1.81	-0.92	-0.70	- 2.43	- 0.79	- 3.62	
		-2.93	-1.92	-0.79	- 3.74	- 1.49		

TABLE 2—Continued

	ANIMAL NUMBER AND SIDE					
	64 Left	65 Right	66 Right	67 Right	68 Right	69 Right
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4th half-turn to 5th half-turn	-0.50	+4.17	+4.96	- 1.97	+ 2.66	- 0.38
	-1.25	+1.46	+2.23	- 3.69	+ 0.86	- 0.34
	-2.22	+0.23	+0.48	- 3.22	+ 0.70	- 0.28
	-2.97	-0.60	-0.38	- 3.70	+ 0.50	- 0.68
	-3.28	-0.82	-0.67	- 3.92	- 0.75	- 0.97
	-5.00	-1.64	-2.63	- 3.84	- 1.01	- 2.39
			- 5.36			
5th half-turn to 6th half-turn	+0.91	+2.88	+2.08	+ 2.27	+ 3.00	- 1.87
	-0.47	+0.21	+0.64	- 1.76	+ 0.76	- 2.56
	-1.64	-1.22	-0.49	- 2.31	- 0.44	- 3.21
	-1.88	-2.30	-1.68	- 3.52	- 1.58	- 3.42
	-2.65	-3.34	-2.61	- 4.40	- 2.85	- 3.94
	-3.63	-5.71	-4.31	- 5.51	- 3.78	- 4.98
			- 6.37			
6th half-turn to 7th half-turn	-0.26	+2.37	-0.79	- 4.87	+ 3.45	+ 3.89
	-2.58	+0.49	-2.67	- 5.61	- 0.56	- 0.10
	-3.73	-1.05	-3.47	- 6.57	- 0.86	- 0.22
	-3.81	-3.06	-5.28	- 7.07	- 1.61	- 2.29
	-5.68	-3.39	-6.16	- 7.35	- 2.68	- 3.73
7th half-turn to 8th half-turn	-2.99	+1.66	-3.18	- 5.43	+ 2.03	+ 4.60
	-4.69	-2.26	-3.43	- 6.58	- 0.83	+ 0.17
	-5.67	-4.00	-4.31	- 8.38	- 4.75	- 0.72
	-5.87	-4.52	-4.92	- 9.69	- 5.72	- 2.43
	-6.99		-5.40	-10.89	- 7.92	
8th half-turn to apical part		-0.33	+6.83	+ 7.04		+ 3.35
		-1.34	+6.20	+ 2.99		- 2.08
		-0.38	0.00	0.00		+ 1.39
		0.00				0.00

more from a circular nature than any other part does, and that the degree of curvature is less than that of a circular arc of the dimensions of that determined by this graphic method. On the other hand, the figures for the second to third half-turns are in each case positive, and the percentage of error much less than for the first to second half-turns; this indicates that in this region the curvature

of the organ of Corti deviated from the graph in the direction of having a slightly greater degree of curvature than the circular arcs of the graph. In the remaining turns there is more variation in the individual cases, but the most common result is that of having the tangential sections with a positive error which grows less and crosses the zero point into negative values toward the modiolar sections.

In the actual use of the method in determining the extent and position of lesions in given cases, a correction factor could be determined and applied for the portions of the given cochlea, which ought to give values accurate enough to enable recognition of any numerical ratios favoring any of the theoretical requirements of the several theories of hearing.

COCHLEAR VARIATIONS

An interesting secondary result of the method is the demonstration of the amount of variation of individual cochleæ, both in total size and in relative proportions of the various parts. This is best brought out by tabulating the number of sections through which the various half-turns extend. Such a tabulation is given in table 3, the figures of the table being the number of sections from one tangential cut of the union of pillar cells of the organ of Corti to the next tangential cut of the same part; except for the vestibular part and the apical part, where the last ones are not cut tangentially. Variations in the orientation of the blocks for sectioning may be the cause for some of this, but the writer thinks that the orientation was too similar in the several series to account for very much of it; and, further, if this were the cause, the variation would be in the same direction for all turns, which it is not. Even the few cases recorded are sufficient to show that it is not at all correlated with the weight of the animals, the figures for which have been included in the tabulations. For instance, the smallest animal, weighing 225 grams, has a cochlea in which the distances between the several adjacent tangential cuts of the union of pillar cells are greater than for the corresponding turns of the largest animal, weighing 350 grams, and the variations are not uniform.

COMPARISON OF VARIOUS RECORDS

To facilitate comparison of the lesions in a large number of cochleae, the positions and lengths determined by this method may be transferred to charts such as the author used in recording the lesions of the detonation deafness experiments. One of these is reproduced here as chart 1 for purposes of illustration. In such a chart the relative lengths given each half-turn are those of an

TABLE 3

Variation in individual cochleae. The figures show the number of 7μ sections included in each turn (see text matter, p. 154, for parts measured)

	WEIGHT OF ANIMAL IN GRAMS						
	320	320	285	340	350	255	225
Animal number and side.....{	64 Left	64 Right	65 Right	66 Right	67 Right	68 Right	69 Right
'Vestibular' part.....	80	80	73	101	74	105	99
1st half-turn.....	314	307	321	329	286	316	287
2nd half-turn.....	265	269	266	293	271	278	281
3rd half-turn.....	199	197	192	201	187	200	198
4th half-turn.....	172	175	176	186	169	185	180
5th half-turn.....	156	148	146	152	140	153	148
6th half-turn.....	145	143	139	146	134	147	140
7th half-turn.....	116	114	113	119	108	118	112
8th half-turn.....	83	84	86	98	87	94	88
Apical part.....	20	21	31	46	14	30	52

average cochlea; so there would be, of course, some slight adjustments necessary in fitting each cochlea to the fixed length, but the gain in facility of comparison might in some cases render it advisable. The original graphic plots can of course be reproduced as either line drawings or halftones.

SUMMARY

Most observers have reported cochlear injuries by written protocols and midmodiolar section diagrams, making only rough estimates of the relative lengths of various lesions, usually in terms of approximate parts of turns, without evaluation of the lengths of various turns. Better interpretation of results is permitted by

the author's former method of charting the organ of Corti as a straight band with the relative lengths of the various half-turns approximated, but since the linear value of the several parts of a curved structure contained in radial, oblique, and tangential sections varies, the locations of the ends of lesions were only approximate. The graphic reconstruction method here presented evaluates directly the approximate lengths of all portions of the organ of Corti when cut in serial sections. On ruled paper each space represents a section; the limiting sections for each half-turn are determined and a series of semicircular arcs drawn connecting the points thus found; the result is a 'spiral-like' curve making approximate allowance for variations in linear values of different sections. This is the 'base-line' for charting any desired data. The percentages of error for different portions have been determined for six graphs and are recorded.

The more accurate data secured by this new method may be transferred to straight-line charts to facilitate comparison of numerous observations. The method will not only yield more accurate data, but will, if adopted by other workers in this field of investigation, also render the results of all available to the others in a form which will permit of more ready comparison than has previously been possible. This would, without question, greatly facilitate the solution of the problem of the physiology of hearing.

The general method may be modified so that other parts of cochlea may also be graphically represented. The demonstration of variation in individual cochleæ is a secondary result.

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Resumen por el autor, Oscar V. Batson.

La coloración diferencial del hueso.

I. El teñido de ejemplares conservados.

El autor ha probado una serie de derivados de la alizarina y mordientes con el propósito de hallar, si esto fuese posible, un colorante que ofrezca una coloración de contraste con la obtenida por el rojo de rubia ordinario. Para los experimentos ha empleado embriones de pollo y cerdo. El material fué fijado en formol al 10 por ciento. Para eliminar el color de la sangre se empleó una solución débil de peróxido de hidrógeno. La solución tintórea fué una solución saturada del colorante en alcohol de 95°, una parte, y veinte partes de alcohol de 95°. Los ejemplares fueron diferenciados en una solución al medio por ciento de ácido sulfúrico en alcohol de 95°, después de lo cual fueron deshidratados y aclarados según el método de Spalteholz. Los veinte colorantes que pudieron obtenerse fueron los siguientes:

Negro de alizarina ácido, R. Meister, Lucius & Brüning	Pardo de alizarina RB, polvo, Fr. Bayer & Co.
Negro de alizarina SR, polvo, Fr. Bayer & Co.	Negro de alizarol 3G, Nat. Aniline and Chemical Co.
Azul de alizarina Br3g, polvo, Fr. Bayer & Co.	Carmín de índigo, Grüber
Negro de alizarina Bayer NR, Polvo, Fr. Bayer & Co.	Ácido carmínico, Grüber
Negro de alizarina SBB, polvo, Fr. Bayer & Co.	Rojo de Burdeos, Grüber
Pardo de alizarina AR, polvo, Fr. Bayer & Co.	Naranja, Grüber

THE DIFFERENTIAL STAINING OF BONE

I. THE STAINING OF PRESERVED SPECIMENS¹

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The study of ossification centers, of healing in fractures, and of bone growth in general has been greatly facilitated by the use of alizarin in its natural and synthetic forms. That madder root when fed to animals colors the bone red has long been known. It seems that Belchier (1736) rediscovered this fact and brought it to popular attention. Almost 150 years earlier, however, Laevinus Lemnius (1581) mentioned this property of madder root.² Considering the numerous editions and translations of Lemnius' "Hidden Miracles of Nature," it seems remarkable that no use was made of this observation.

Biologists and chemists have examined madder root to find the active staining principles. The chief of these have been found to be alizarin and purpurin, mainly the former. Since the pure synthetic alizarin has been available it has been largely used in the staining of preserved specimens, for the demonstration of the skeleton. This staining technic coupled with clearing methods has been presented in detail by Lundvall ('04, '05) and Spalteholz ('14). Alizarin in the form of its soluble salt, sodium alizarin monosulphonate, has also been used to supplant madder root in the vital staining of bone. Gottlieb ('14), in a very thorough paper, describes the use of the above salt for vital staining. Brooks ('17, '20) more recently has reported a series

¹A series of specimens stained with the described dyes was demonstrated at the meetings of the American Association of Anatomists at The Wistar Institute, Philadelphia, March, 1921. This demonstration included a set that had been prepared in August, 1920.

²" . . . erythrodanum seu rubea, qua ossa pecudum sandicino rubentique colore imbut, . . ." De Miraculis Occultis Naturae, p. 390, lines 30 and 31.

of studies in which the same salt was used, and it is quite probable that sodium alizarin monosulphonate is now used in most laboratories for vital staining of bone.

Graebe and Lieberman first produced alizarin synthetically in 1868. Since that time many synthetic alizarin derivatives have been produced by the dye chemists. A glance at the catalogs of the dye firms reveals alizarin reds, browns, blacks, blues, greens, and yellows. Apparently none of these have been used for the successful staining of bone. Ehrlich ('85) used alizarin blue S in studying oxidation in tissues, but with this stain did not report any staining of bone. Rawitz ('96) who used alizarin extensively in microscopic technic did not mention bone staining. Spalteholz ('14) uses small quantities of alizarin cyanatum in staining preserved specimens to improve the color of the alizarin red. Gottlieb ('14) unsuccessfully tried to stain bone *intra vitam* with alizarin blue and alizarin green (brand or formula not mentioned).³ In reporting Bardeen's technic for the study of ossification centers, Hill ('06) describes the former's use of alum carmine, a dye of a wholly different chemical series. Miller ('21) in a recent paper refers to the same work.

In certain types of bone work a stain affording a contrast to the red-brown of the usual alizarin would seem desirable. It might be possible to alternate the red with a contrasting color in *intra vitam* work, or it might be possible to bring out more detail after preservation by the use of a counterstain for that part of the bone not colored during life. With the view of possibly finding such a stain, the following study of alizarin derivatives and mordant dyes was begun.

Among the readily soluble alizarin cloth dyes available those were selected which gave promise of affording a contrast to the alizarin red. The non-alizarin mordant dyes of the histological laboratory were tried out, partially to see if the anthraquinone grouping (present in the alizarins) is an essential element of a bone stain and partially in the hope of finding a more brilliant bone stain.

³ "Ich möchte hier noch kurz erwähnen, dass ich mit einer Reihe verwandter Farbstoffe (Alizarinblau und Alizarin grün) nach dieser Richtung Versuche angestellt habe, sämtlich aber negativ ausgefallen sind" (S. 192).

In investigating the properties of the various stains the following technic was used. The tissue consisted of portions of pig embryos ranging in size from 3.5 cm. to 8 cm. These embryos were part of the laboratory stock obtained from the packing house fixed in 10 per cent liquor formaldehyde. The blood color was removed from part of the material by bleaching in a $\frac{1}{2}$ per cent by volume solution of hydrogen peroxide (the ordinary peroxide of commerce is 3 per cent by volume), followed by removing the gas bubbles with the suction pump.

Stock solution of stain

Strain being investigated.....	250 mg.
Water.....	5 cc.
Shake well and add of 95 per cent alcohol.....	95 cc.

In no case did all of the stain go into solution.

1. Material washed in water.
2. Stained twenty-four to forty-eight hours in

Stock solution.....	1 part
95 per cent alcohol.....	20 parts

3. Differentiated in $\frac{1}{2}$ per cent solution of sulphuric acid in 95 per cent alcohol.

4. Dehydrated and cleared after the method of Spalteholz.

To the present time twelve stains have been found to combine differentially with the bony skeletal elements. A list of the stains, with a brief comment on their behavior and the results obtained, is given below.

Alizarin group

1. Acid alizarin black R. Farbwerke vorm. Meister, Lucius, & Brüning. Hoechst o/M. Stains the skeletal elements a deep reddish purple. The soft tissues and cartilages decolorize rather slowly; however, differentiation is good from the beginning.

2. Alizarin-black SR powder. Farben fabriken vorm. Fr. Bayer & Co., Elberfeld. This stain likewise imparts a purple color to the bones. Satisfactory stains may be obtained from 1 to 250 dilutions. When this is done the bone is not stained so intensely, but decolorization is easier. Must be differentiated in sulphuric acid alcohol.

3. Alizarin-blue Br3G powder. Bayer. Stain is fairly differential from the start. Bone is an azure blue.

4. Alizarin-black-Bayer NR powder. Bayer. This stain gives a good purple to the bones. The other tissues decolorize readily and the end-result is a very sharp stain. See no. 8.

5. Alizarin black SBB powder. Bayer. Bones a sharp indigo. The other tissues take this stain slightly. The differentiation is rapid and sharp. This stain seems to be one of the most satisfactory. Its color is devoid of reddish tinge.

6. Alizarin brown A R powder. Bayer. Imparts a reddish brown to bone.

7. Alizarin brown R B powder. Bayer. Very similar to no. 6.

8. Alizarol black 3 G. National Analine and Chemical Co., New York. A new American stain, supposedly an alizarin. The shade is slightly different from no. 4, but otherwise the dye is quite similar. This is a very satisfactory stain. It is available in quantities.

9. Indigocarmine, Grübler. This stain can be used in a saturated solution or in any dilution. It decolorizes perfectly in water as well as in the acid alcohol mentioned. The resulting stain is a medium indigo. One of the easiest stains of the series to handle and one producing an excellent preparation.

10. Carminic acid, Grübler. This stain like the previous one can be differentiated in water. If the reaction of the mounting fluid is acid the color of the bone in the specimen is an orange-red, if the reaction is alkaline the color approaches a Bordeaux. If acid decolorization is used the specimen should be alkalinized before mounting. Dr. Barden's use of alum carmine was referred to earlier. It is perfectly obvious that the use of carminic acid is but a modification of this technic.

11. Bordeaux red, Grübler. This stain can be used in a saturated solution if desired, but this makes decolorization a little more tedious. The skeletal elements come out a brilliant shade of Bordeaux and if decolorized properly the rest of the structures are colorless. The end-results seems much better than those obtained with the usual alizarin red.

12. Aurantia, Grübler. Solutions near saturation work best. The bones stain a bright orange-yellow. The crystalline stain gives a deeper stain than the amorphous.

In working with the individual stains certain modifications as noted above were found to be desirable, but on the whole the standard technic was entirely satisfactory for the preliminary work. Portions of the above specimens were subjected to treatment with 5 per cent sulphuric acid in 95 per cent alcohol to test the permanence of the stains. Some decolorization (not decalcification) was noted when the time in the 5 per cent acid was extended over forty-eight hours. This permanence to sulphuric acid applies to the skeleton of the chick at hatching and to the

skeletons of pig embryos of the stages mentioned. Sulphuric acid has not been satisfactory when used on the bone of young rats. Lundval ('05) must have had in mind the acids that form soluble calcium salts when he comments, "Da das Alizarin in sauren Lösungen beinahe vollständig entfärbt wird, kann man keine von den schon beschriebenden Knorpelfarbenmethoden verwenden." Calcium sulphate is quite insoluble. It should be noted here that in differentiating specimens stained by sodium alizarin monosulphonate, the acidulation of the alcohol with sulphuric acid causes the red soft tissues to become immediately yellow, while the bone remains red. This yellow color is readily extractable. Spalteholz ('14) made use of these facts in his earlier technic by adding small amounts of acetic acid to the staining solution itself.

SUMMARY

Of these new bone stains indigo-carmin, Bordeaux red, alizarol black 3G (National Aniline and Chemical Co.) and alizarine black 8BB powder (Bayer), seem particularly suitable for staining preserved specimens.

The number of stains shown to combine differentially with bone indicates that there is a large number of mordant or adjective dyes with this property.

The anthraquinone grouping is not necessary for bone staining.

Weak solutions of sulphuric acid are adequate and satisfactory decolorizing agents for soft tissues in differentiating bone stains.

Staining of bone in preserved specimens is largely a matter of differential decolorization.

I wish to thank Dr. A. G. Pohlman, of St. Louis University, in whose laboratory the preliminary experiments for this paper were carried out, and to acknowledge especially my indebtedness to Prof. R. Fischer, of the Department of Chemistry of this University, who placed a collection of alizarines at my disposal.

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Resumen por el autor, Oscar V. Batson.

La restauración del material anatómico momificado.

El material anatómico que se ha secado demasiado para la disección puede restaurarse completamente al estado fresco mediante inyección subcutánea de un líquido conservador. El líquido empleado, cuya composición no es de gran importancia en tanto que contenga una gran cantidad de agua, debe inyectarse primero en la tela subcutánea y después puede inyectarse debajo de las fascias musculares. La piel se ablanda rápidamente, y con ayuda del masaje al cabo de unos minutos aparece casi como en el vivo. Los antisépticos que persisten en el cadáver desde el previo embalsamamiento no son extraídos de los órganos, como sucede cuando se emplea el método ordinario de remojar el material seco.

Translation by José F. Nonidez
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RESTORING MUMMIFIED ANATOMICAL MATERIAL.

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ONE FIGURE

Gross anatomical material is difficult to keep in good condition for dissection, even with frequent attention. This is particularly true of the hands and feet and of any region marked by bony prominences. This drying is prone to occur during storage and during holiday recesses despite precautions.

The methods commonly used to combat this evil are either to immerse the member in a weak antiseptic fluid or to cover it with cloths thoroughly saturated with such a fluid. These procedures have two disadvantages: The skin is fairly impervious to the fluid, particularly if it has dried to the point where it is board-like and translucent; either of the above methods tend to remove the preservative used in embalming in direct proportion to their efficacy in softening.

These disadvantages may be entirely overcome and the most hardened, translucent, mummified hand or foot completely restored by combining massage with the subcutaneous injection of fluid. A 25-cc. or 50-cc. metal barrel syringe, with a large needle, seems to be the most satisfactory instrument. The pressure bottle does not give as good results. The needle is passed into some fairly areolar region, and some 10 cc. of fluid injected. This softens the tissues for a small area, and by beginning at the border of this for the next injection, and by massaging the fluid around in the subcutaneous tissues the work is gradually extended. The skin softens readily when the fluid is applied from below and in a few minutes looks even life-like. The contained antiseptics remaining from the original embalming are not washed out. If the fluid injected contains a large percentage of water, the exact

nature seems immaterial. The following fluid works well and seems to aid in preventing a recurrence of drying:

Gum tragacanth.....	5 grams
Glycerine.....	100 cc.
Water up to.....	1 liter

Let stand 48 hours and filter through gauze.

The muscles, fascias, and skin can be completely restored. Material which has been preserved with a large percentage of liquor formaldehyde does not revive so completely.



Fig. 1 The ulnar side of the forearm and hand together with the ring- and little fingers has received subcutaneous injections of the fluid recommended. The remainder of the hand is representative of the condition of the whole hand before injection.

Resumen por el autor, H. D. Senior.

La arteria comitans nervi peronaei communis.

El autor no ha podido comprobar la observación de Salvi, hecha al describir por primera vez esta arteria en 1899, con referencia a la constancia de la existencia de esta arteria en el miembro adulto. En su opinión, la arteria comitans nervi peronaei communis falta más a menudo que existe. La arteria nervi peronaei communis embrionaria descrita por De Vriese en 1902 y considerada por dicho autor como precursora de la del adulto ha sido hallada por el autor una sola vez al examinar un número relativamente elevado de miembros embrionarios con el fin de encontrarla. Cuando existe, esta arteria puede probablemente llevar a cabo, durante algún estado del desarrollo, la función asumida definitivamente en casos normales por la parte proximal de la arteria tibial anterior; esto parece haber sucedido, en efecto, en un miembro mencionado por Velpeau en 1835. La opinión de De Vriese, sin embargo, sobre la constancia de la aparición de esta arteria embrionaria y su participación en el desarrollo normal de la arteria tibial anterior parece haberse basado en el exámen de material embrionario imperfectamente conservado. La opinión ulterior expresado por dicho autor, y mantenida por Manno en 1906, con referencia a la identidad de la arterias inferolateral de la rodilla y la recurrente tibial anterior del adulto y la arteria nervi peronaei communis del embrión no parece posible bajo el punto de vista anatómico.

Translation by José F. Nonidez
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THE ARTERIA COMITANS NERVI PERONAEI COMMUNIS

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LITERATURE

In 1899 G. Salvi described and figured a branch of the popliteal artery that accompanies the common peroneal and the proximal part of the deep peroneal nerve and ends by joining the a. tibialis anterior. Salvi's description of the a. comitans nervi peronaei seems the first to have been given, although the proximal part of an abnormal anterior tibial artery referred to very briefly by Velpeau in 1835 may quite conceivably have been a vessel of the same nature.¹ Further evidence of the occasional existence of a common peroneal artery is to be found also among the numerous illustrations of dissections of the lower extremity that were published during the last century.²

In the particular case described by Salvi the a. comitans nervi peronaei communis arose from a branch of the popliteal artery from which also an a. saphena parva (or a. suralis magna) took origin. Becoming free about the level of the superior articular surface of the tibia, the a. comitans followed the course of the common peroneal nerve to the neck of the fibula and then accompanied the deep peroneal as far as the junction of the

¹ "Je l'ai vue (l'artère tibialis anterior) deux fois devenir superficielle des le milieu de la jambe. Dans l'un des ces cas, elle sortait comme à l'ordinaire de la poplitée. Dans l'autre, au lieu de traverser le ligament interosseux, elle se contournait en dehors du péroné et suivait le trajet du nerf musculo-cutané." Velpeau ('35), p. 44.

It is unfortunate that no specific mention is made of the relation borne by the proximal part of the artery of the second case to the common peroneal nerve.

² See, for example, Quain ('44), pl. 78, fig. 1; Hyrtl ('66), taf. 3, fig. 3; Ellis and Ford ('76), pl. 53.

proximal and middle thirds of the tibia, where it joined the anterior tibial artery. The *a. comitans nervi peronei* gave origin to two long slender branches: the first, arising opposite the neck of the fibula, followed the lateral aspect of that bone to the lateral malleolus, where it anastomosed with the other arteries in the neighborhood. The second, arising near the terminal end of the parent vessel, became the *a. comitans nervi peronei superficialis*, which was also described for the first time in this publication. The case described in Salvi's paper is illustrated by his figures 3 and 4; figure 1 illustrates a vessel of an almost precisely similar nature that occurred in another limb in which the *a. saphena magna* was present also. The author states that the accompanying arteries of the peroneal nerves are present in a more or less well-developed state in all individuals (p. 37).

Since the publication of Salvi's paper, Manno has described *arteriae comitantes* of the peroneal nerves as occurring in five other human lower extremities. In 1906 the latter author described an adult limb in which the ischiadic artery had persisted and in which the common peroneal artery was present also. In the same year he published an extended study of the relations of the common peroneal artery of vertebrates in general, which contained descriptions of the peroneal *arteriae comitantes* of four other human limbs. The so-called common peroneal artery illustrated in figure 2 of the latter paper appears to be nothing more than an unusually large anastomotic connection between the *a. genu inferior lateralis* and the *a. recurrens tibialis anterior*, and is described as such.³ The peroneal *arteriae comitantes* of the other cases seem to resemble closely those described by Salvi.

In connection with Manno's identification of the vessel illustrated in the figure just referred to, it may be remarked that, inasmuch as the inferior lateral genicular artery of the adult

³ "Nell' arto addominale destro di un bambino di un mese, che ho riprodotto nella figura 2 si trova un'arteria peronea communis molto evidente formata dall' anastomosi di un ramo discendente dell' arteria genu lateralis inferior e di un ramo ascendente dell' arteria recurrens tibialis antica. Essa decorre parallela al nervus peroneus communis, ma un pò al di sopra di questo." Manno ('06 a), p. 273.

limb is separated from the common peroneal nerve by the lateral head of the gastrocnemius muscle and by the plantaris, it cannot be accepted as the equivalent of the proximal part of the a. comitans nervi peronei communis described by Salvi. The fact that the channel of connection between the popliteal and anterior tibial arteries formed by the anastomosis between the a. genu lateralis inferior and the a. recurrens tibialis anterior pursues a course entirely different from that followed by the a. comitans nervi peronei communis, is brought out clearly by Salvi's figures 1 and 4, in which the two channels of connection between the popliteal and the anterior artery are shown to coexist. Manno is not only willing to accept the a. genu inferior lateralis as representing the proximal part of the a. comitans nervi peronei communis, but seems willing also to accept the a. propria articularis capituli fibulae of Weber in a somewhat similar light.⁴ The latter artery has very little in common with the a. comitans nervi peronei communis, for Weber's description indicates quite clearly that it is separated from the common peroneal nerve not only by the gastrocnemius and plantaris muscles, but also by the soleus.⁵

DeVriese's conception of the embryonic history of the a. comitans nervi peronei communis is set forth in the description of the development of the arteries of the human extremities, published by that author in 1902. The occurrence of the a. comitans nervi peronei communis in the adult limb is described as resulting from the persistence of an embryonic artery named the a. nervi peronei communis (p. 719). The a. nervi peronei communis is described as a branch of the a. ischiadica that follows the course of the common peroneal nerve and per-

⁴ "Quindi l'arteria capituli fibulae di Weber, sia per l'origine sua dalla poplitea, che per il tragitto parallelo al nervo peroneo communis, è da considerare piuttosto come una ridotto arteria comitans nervi peronei communis." Manno ('06 a), p. 275.

⁵ Weber described the a. articularis propria capituli fibulae in the following terms ('42, p. 207): "Sie ist etwa $\frac{1}{2}$ " dick, entspringt am Ende der A. poplitea, und tritt quer nach aussen zum Köpfchen des Wadenbeins, und verläuft von dem äussern Kopf des M. soleus und dem Ursprungstheil des M. peroneus longus und Extensor digitorum communis longus bedekt," u. s. w.

forms the function of transmitting blood to the embryonic *a. nervi peronaei profundi*, which becomes the distal part of the adult *a. tibialis anterior*. At about the stage of 20 mm., this function having been assumed by the embryonic artery that becomes the proximal part of the definitive anterior tibial artery, the *a. nervi peronaei communis* is described as being reduced in size, but as persisting in the form of the *a. genu inferior lateralis*, the *a. recurrens tibialis anterior* and the anastomosis between them.⁶

There is no difficulty in understanding how an embryonic artery that follows the course of the embryonic common peroneal nerve, as does the *a. nervi peronaei communis* of DeVriese, might persist in adult life as the *a. comitans nervi peronaei communis*. It would seem impossible, however, that such an artery should become transformed into the *a. genu inferior lateralis* and the *a. recurrens tibialis anterior* of the normal adult limb.⁶ The anatomical considerations militating against the acceptance of such an identification have been referred to in connection with one of the arteries described by Manno as an example of the *a. comitans nervi peronaei communis*.

DEVELOPMENT

That an *a. comitans nervi peronaei communis* occurs in some embryonic limbs there cannot be the slightest doubt, for the literature contains incontestable evidence of the existence of such an artery in the adult. The question, however, as to whether the artery is constantly present, either in the adult or in the embryo, is one which such personal experience as I have had would lead me to answer in the negative. As far as the adult limb is concerned, my information has been derived solely from the ordinary run of dissecting-room material, and in such material I have encountered the artery very rarely. This experience, however, is exactly what I should expect from the

⁶ "La partie proximale de l'art. nervi peronaei s'atrophie mais persiste chez l'adulte comme arter. recurrens tibialis anterior et plus haute, arteria genu inferior lateralis; l'arteria nervi peronaei profundi est l'arteria tibialis anterior de l'anatomie humaine." DeVriese ('02), p. 695.

result of an examination of a good deal of embryonic material, which has been mainly negative in character. Although I have examined the popliteal and sural arteries of many of the more fully developed embryos belonging to the collection of the Carnegie Institution, and the deep popliteal artery of many of those in earlier stages of development, I have not been able to find a branch of any of these arteries that follows the course of the *nervus peronaeus communis*. A small *a. comitans* may be overlooked occasionally perhaps in examining serial sections, but I am quite certain that no such branch has been present in any of the twelve tolerably well-preserved embryonic human lower limbs of which I have reconstructed all the arteries. I know of only two embryonic arteries that follow any part of the course of the common peroneal nerve; one of these was found in the right lower extremity of an embryo of 15 mm. (no. 350 of the Carnegie Institute Collection), and the other in the left lower extremity of one of 12 mm. (no. 3 of the Cornell Collection).⁷ All the arteries of both lower limbs of these two embryos were reconstructed; the vessel under consideration occurs in one limb only.

The common peroneal artery of the 15-mm. embryo proves to be nothing more than a large branch of the anterior tibial recurrent that accompanies the common peroneal nerve as far as the upper border of the lateral condyle of the femur. It is not sufficiently long to reach the *a. poplitea profunda* and seems to have very little in common with the adult *a. comitans nervi peronaei communis*. In all probability it would have figured in adult life as an unusually large branch of the anterior tibial recurrent artery. The common peroneal artery of the 12-mm. Cornell embryo is very much more suggestive, although in the present state of our knowledge the nature of its future course of development is a matter of pure speculation. In view, however, of the position occupied by the ischiadic and common peroneal

⁷ I am greatly indebted to Prof. C. R. Stockard, of Cornell Medical School, New York, N. Y., and to Prof. George L. Streeter, of the Carnegie Laboratory of Embryology, Baltimore, Md., for their courtesy in allowing me to use these embryos for study.

arteries with respect to the tibial and common peroneal nerves, it seems not unlikely that the vessel in question may prove to be an example of the embryonic precursor of the adult artery described by Salvi.

In the 12-mm. embryo in which this vessel occurs, the tibial and common peroneal nerves, which in earlier stages of development diverge sharply from one another as they arise from the sacral plexus, run a parallel course through the proximal third of the thigh, and the ischiadic artery occupies the narrow interval between them. The artery then follows the lateral aspect of the tibial nerve in the usual manner, nearly as far as the level of the knee-joint. Just as the tibial and peroneal nerves of the left limb are beginning to diverge, which they do at a progressively increasing angle, the ischiadic artery gives origin to a common peroneal branch. The latter is an artery of rather large caliber, which, having followed the medial aspect of the common peroneal nerve for some distance, ends abruptly or possibly becomes collapsed at about the junction of the middle and distal thirds of the thigh. It gives origin to a few small branches that enter into the formation of a plexus near the anterior aspect of the ischiadic vein, and to a larger branch that runs for a short distance on the anterior surface of the common peroneal nerve. Since this particular common peroneal artery does not enter the anterior crural region, it cannot be regarded as the precursor of a fully developed *a. comitans nervi peronei communis*, unless it be assumed that it would have done so if the embryo had not perished. The actual length of the artery in the earlier stages of development is not a matter of great importance in the present connection, for Salvi refers to imperfectly developed examples of the adult artery. No representations of imperfectly developed examples of the artery are presented by that author, but it is probable that such a vessel is exemplified in the lateral branch of the popliteal of Barkow's plate 57, figure 1 ('68) and in the lateral branch of the sural artery illustrated in figure 99 of Henle's *Gefässlehre*.

Manno's case, in which the presence of the *a. comitans nervi peronei communis* is associated with persistence of the ischiadic artery in adult life, is most instructive in suggesting the means

by which a common peroneal artery originally taking origin from the *a. ischiadica* might figure in adult life as a branch of the popliteal artery. The relations illustrated in Manno's figure 1 do not differ materially from those encountered usually in embryos of 18 mm., except that the tibial and common peroneal nerves run a parallel course nearly as far distally as the superior angle of the popliteal fossa as they do normally in the adult. The ischiadic artery occupies the narrow interval between the two nerves, and the *a. comitans nervi peronei communis* takes origin from the *a. poplitea*. The figure illustrates rather strikingly the influence that might be exerted by the gradual approximation of the tibial and common peroneal nerves of the 12-mm. embryo under consideration, upon the definitive mutual relation of the two arteries contained in the angular interval between them. The gradual obliteration of the space between the two nerves would almost certainly have initiated a process of progressive union between the ischiadic artery and its common peroneal branch which would have produced an effect of distal migration of the site of origin of the latter vessel. If this process had extended beyond the region of the hiatus tendineus, the origin of the common peroneal branch would automatically have been transferred from the ischiadic to the part of the deep popliteal artery that forms the proximal part of the adult *a. poplitea*. If a common peroneal branch of the ischiadic artery such as that described above should have failed to undergo the migration here suggested before the stage of 19 mm., it might be expected to disappear altogether to become converted into a common peroneal branch of one of the adult *aa. perforantes*.

It is scarcely safe to assume that the *a. comitans nervi peronei communis* of Manno's case arose at first from the *a. ischiadica* and became transferred to the popliteal artery in the manner suggested or that the definitive site of origin of that vessel is usually determined by a process of embryonic migration. It seems not improbable, however, that migration may be one of the regular events in the developmental history of the adult artery described by Salvi, and until such a branch of the popliteal artery, or of the part of the deep popliteal artery that enters

into the composition of that vessel, shall have been found in the embryo, it would be unwise to disregard the possible significance of a common peroneal branch of the ischiadic artery.

I have been greatly interested in the *a. nervi peronei communis* described by DeVriese, both in connection with its apparent similarity to the vessel described here as a somewhat doubtful example of the embryonic precursor of the *a. comitans nervi peronei communis* and on account of the important part it is said to play in the development of the *a. tibialis anterior* and its anterior recurrent branch, and the *a. genu inferior lateralis*. I do not believe that a common peroneal branch of the ischiadic artery, which appears so rarely in the embryonic limb, can take part in the normal development of the adult arteries just mentioned.⁸ On this account I have examined the evidence presented by DeVriese in order to ascertain, if possible, whether the *a. nervi peronei communis* was actually present in any of the embryonic limbs examined by that author; the result of this examination has not been at all convincing.

Sections from the lower extremities of embryos of 10, 13, 16, 20 and 27 mm., respectively, are represented. No artery accompanies the part of the common peroneal nerve shown in figure 23, taken from an embryo of 27 mm., and no sections from suitable levels of the embryos of 16 and 20 mm. have been reproduced. The figures representing sections of the 10- and 13-mm. embryos seem to bear unmistakable evidence of the inadequacy of the preservation of the material from which they were drawn. The ischiadic artery, for instance, seems to have been unrecognizable in the section reproduced as figure 15. It should, no doubt, have occupied the interval between the tibial and common peroneal nerves, as it is shown to do in figures 20 and 21, which represent sections of 16- and 20-mm. embryos, respectively. The wide lacuna around the tibial and common peroneal nerves depicted in figure 15, which is labeled '*a. ischiadica*,' appears to be an

⁸ The manner in which I believe the *a. tibialis anterior* and the *a. recurrens tibialis anterior* to be developed is described on pages 92 and 93 of the "Development of the arteries of the human lower extremity," *Am. Jour. Anat.*, vol. 25. The *a. genu inferior lateralis* is present in embryos of about 23 mm.

artifact, and the same may be said of the lacuna shown to surround the common peroneal nerve of figure 16.⁹

The author's conception of the relation borne by the 'lacune vasculo-nerveuse' of inadequately fixed human material to the vascular plexus, described as ramifying upon all the nerves of the embryonic mammalian extremities, is stated clearly on pages 675 and 676. The opinion that the dimensions of perineural lacunae of this kind are proportional to the number and size of the vessels destroyed in their production (p. 676) is one, however, that may be regarded as open to question. I have been unable to detect the presence of perineural lacunae in perfectly fixed mammalian embryonic material, human or otherwise, and, to judge from the appearance presented by figures 20 to 23, they do not seem to have occurred in the older embryos used by DeVriese. Owing to the nature of the circumstances under which mammalian embryos are obtained for fixation, perineural spaces are to be observed more commonly around the nerves appearing in serial sections of human embryos than around those seen in sections of other embryonic mammals. Considered in conjunction with other evidences of maceration of the tissues prior to fixation, the dimensions of such lacunae seem to me to afford a fair general indication of the degree of unreliability of any material in which they occur for the study of arterial development.

It becomes clear upon studying DeVriese's figures, that two classes of spaces have been labeled as arteries, namely, sections through arteries recognizable as such and sections through perineural lacunae. Under the circumstances referred to in the preceding paragraph, I see no reason for accepting the latter as equivalent to perineural arterial plexuses, even in the conventional sense noted on page 676. I find also, upon leaving out of consideration the perineural spaces that have been labeled as arteries, that all the arteries encountered usually in the developmental stages illustrated are still represented in the figures. I find it extremely difficult on this account to escape the conclusion

⁹ Both Elze ('07) and Evans ('12) have questioned the adequacy of the preservation of the material used by DeVriese.

that the *a. nervi peronaei profundi* of figure 16 and the *a. nervi peronaei superficialis* of figure 17 are not arteries, either in being or in process of development. Having been led into a somewhat similar error of interpretation through the study of poorly preserved material, I venture to offer this criticism of another's work in a spirit of sympathetic appreciation of the difficulties involved.

CONCLUSIONS

1. That the literature relating to the *arteria comitans nervi peronaei communis* is apt to convey an erroneous impression as to the completeness of our knowledge both of the adult vessel and of its embryonic precursor.

2. That the *comitans nervi peronaei communis* is not to be found in all adults, as has been stated by Salvi.

3. That the *a. nervi peronaei communis* described by DeVriese does not take part in the normal development of the *a. genu inferior lateralis*, the *a. recurrens tibialis anterior*, and the *a. tibialis anterior*, as stated by that author, although a branch of the ischiadic artery to which this name might be given is known to accompany the common peroneal nerve of some embryonic limbs.

4. That it is doubtful whether an unquestionable example of the embryonic precursor of the *a. comitans nervi peronaei communis* has ever been described, either in this paper or elsewhere.

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Resumen por el autor, Wilbert A. Clemens.

Un caso de hermafroditismo completo en la rana toro (*Rana catesbiana*).

El ejemplar objeto de este trabajo fué descubierto durante una disección en los trabajos de laboratorio llevados a cabo por los alumnos. Presenta los siguientes caracteres masculinos: Un testículo izquierdo que contiene espermatozoides (con toda probabilidad existía también originariamente un testículo en el lado derecho); vesículas seminales pares, sacos vocales pares, y callos sexuales en ambos pulgares. También presentaba los siguientes caracteres femeninos: Ovarios pares que contienen óvulos y oviductos en ambos lados del cuerpo. Parece indudable que esta rana poseía órganos reproductores masculinos y femeninos completos y funcionales.

Translation by José F. Nonidez
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A CASE OF COMPLETE HERMAPHRODITISM IN A BULLFROG (*RANA CATESBIANA*)

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ONE FIGURE

During the spring of 1919, the writer discovered a case of complete hermaphroditism in a bullfrog (*Rana catesbiana* Shaw). The specimen was under dissection in a laboratory class and unfortunately was not noticed until after some of the viscera had been removed. A considerable number of cases of various degrees of hermaphroditism have been reported in European Anura, but apparently the only previously described case in American Anura is that of an individual of *Bufo lentiginosus* (King, '10).¹ The case here reported is therefore remarkable when one considers the number of frogs dissected annually in laboratory classes.

The origin of the specimen is not known definitely, but the supply of frogs for that year and for the previous five years came from Keene, Ontario, from a dealer who maintained a frog pond there, and it is likely that the frog was produced at that place. The frogs were usually received about the middle of September.

The specimen is an adult of large size, as is shown by the following measurements: length from anus to tip of snout, 14.5 cm.; width of head at posterior angles of jaws, 5.8 cm.; width of tympanic membrane, 1.7 cm.; length of extended posterior right leg from anus to tip of fourth digit, 20.4 cm.

Externally there is nothing abnormal in the appearance of the specimen except that the shape of the body somewhat suggests

¹ King, Helen D. 1910 Some anomalies in the genital organs of *Bufo lentiginosus* and their probable significance. *Am. Jour. Anat.*, vol. 10, p. 159.

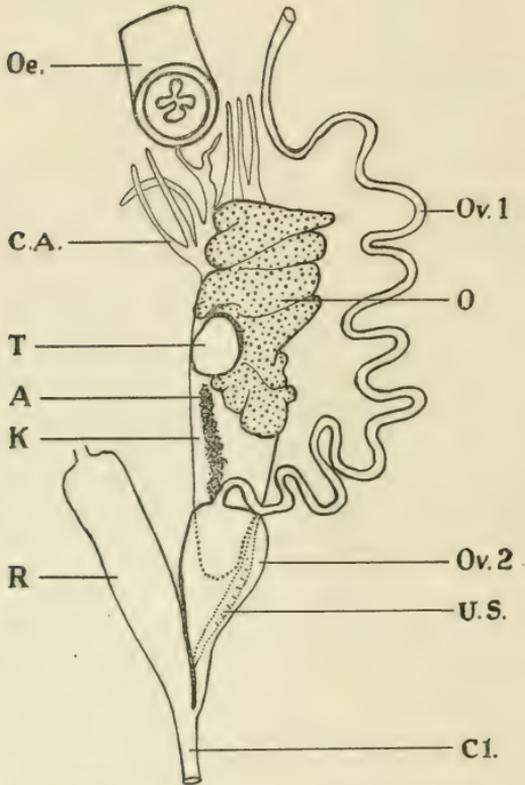


Fig. 1 Semidiagrammatic drawing of a ventral view of the reproductive organs of left side of an hermaphroditic bullfrog (*Rana catesbiana* Shaw). Natural size. A, adrenal glands; Cl, cloaca; C.A., corpora adiposa; K, kidney; O, ovary; Oe, oesophagus; Ov₁, convoluted portion of oviduct; Ov₂, enlarged posterior portion of oviduct with posterior end of kidney and ureter and seminal vesicle dorsal to it; R, rectum; T, testis; U.S., ureter and seminal vesicle.

that of a female, while the presence of pads on the thumbs indicates maleness. The vocal sacs and their openings into the mouth cavity are well developed.

Internally all the organs and organ systems were normally developed except the reproductive system. The student removed the respiratory system, the major portion of the digestive system, the heart and many blood vessels, the right ovary, and a portion of the right oviduct in the course of the dissection. His drawings, however, of these systems did not show anything abnormal. On the left side there is a typical ovary, fan shaped when spread out, having a length along the outer margin of approximately 10 cm. and a width of 2 cm. The ova can be seen with the naked eye and average about 0.5 mm. in diameter, although one was observed which had a diameter of 1.3 mm. and showed light and dark poles. These ova are thus about the normal size for this species for September, when the frog was probably killed. In the lower part of the ovary is embedded a testis approximately 6×8 mm. It contains fully developed spermatozoa. Vasa efferentia cannot be accurately identified and possibly have been destroyed. The student stated that there had been an ovary on the right side, but whether there was also a testis is not known. However, well-developed seminal vesicles are present on the ureters of both sides, which would seem to be good evidence for the presence of paired testes.

SUMMARY

Male characters

A left testis, containing spermatozoa (doubtless a right testis was also present originally).

Paired seminal vesicles.

Paired vocal sacs.

Paired thumb pads.

Female characters

Paired ovaries, containing eggs.

Paired oviducts.

There would seem to be no doubt, therefore, that this frog had complete and functional paired sets of both male and female reproductive organs.

Resumen por el autor, F. H. Swett.

Posición invertida de las vísceras en mónstruos dobles de trucha.

El autor describe brevemente en el presente trabajo quince embriones dobles de trucha con especial mención de la disposición de sus vísceras. En nueve casos la posición de las vísceras de ambos componentes es normal; en uno, la de A (el gemelo del lado derecho) está invertida; en dos, la posición de las vísceras de B (el gemelo del lado izquierdo) está invertida; y en tres casos B es normal y A presenta una posición visceral indeterminada.

Hay algunas indicaciones sobre la existencia de una correlación general entre el grado de duplicación y la presencia de una posición visceral invertida, pero la significación de dicha correlación es dudosa. Los gemelos de tipo parásito pueden presentar posición visceral invertida. Dicha posición puede presentarse en cualquiera de los gemelos, si bien en general aparece más a menudo en el del lado derecho (componente A).

Translation by José F. Nonidez
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SITUS INVERSUS VISCERUM IN DOUBLE TROUT

F. H. SWETT

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SIX FIGURES

This investigation on situs viscerum was undertaken at the suggestion of Professor Harrison, to whom the writer gladly acknowledges his indebtedness for helpful criticism and advice. The material consists of a small collection of trout embryos (*Salmo fario*) taken by Prof. A. Petrunkevitch in Freiburg in 1901, a number of which show abnormalities of different kinds. The results are presented in the hope that they may prove of value in supplementing previous observations, particularly those of Morrill ('19), who made use of similar material. For this reason, then, very little attempt has been made to explain the extraordinary conditions of the viscera; they are merely described briefly and the relationship of the degree of external and internal doubling to the occurrence of situs inversus, in so far as it is disclosed by these animals, is shown.

From these trout embryos, fifteen which showed doubling to a greater or less degree were isolated. The specimens had been fixed in corrosive sublimate and preserved in 70 per cent alcohol, and it was found necessary to run them back to water before the yolk mass, which in each case united the two components, could be dissected off without damage to the underlying tissues. The region of fusion of the vertebral columns is taken as the criterion of the amount of external doubling, that of the intestinal tracts of internal doubling. The embryos show a fairly complete gradation in amount of doubling from almost total separation of the vertebral columns to a very close union as parasite and autosite. These terms are used to denote, respectively, the smaller and larger of the two unequal compo-

nents of the same monster. In every case one abdominal cavity is common to the two components, the ventral body walls being fused around a common yolk mass. In some cases there is such distortion of the viscera that it is almost impossible to decide whether the situs is normal or not. The asymmetry of the hearts could not be made out with certainty. The dissections were all made under the low power of the binocular and the drawings are from camera-lucida sketches of these dissections (magnification, $\times 8$.) The nomenclature adopted by Morrill ('19) for the components of the monsters is followed in each case i.e., designating as A the right twin (at the observer's left in a ventral view), and as B the left twin (right from the ventral aspect). The components are thus referred to in the descriptions, table, and figures.

MORPHOLOGICAL FINDINGS

The embryos are arranged in this section in the order of their degree of external doubling.

No. 1. The components of this monster are united by their ventral surfaces so that their median planes are almost coincident. Component A is slightly larger than B, showing a higher degree of development. The right eye of A is normal, the left has a rudimentary lens, as does the right eye of B, while the left eye of B is not visible externally. The vertebral columns of both components are separate to near the base of the tail, where the body musculature fuses more completely, forming a single tail. There are, however, two dorsal and two caudal fins. The abdominal cavity contains the viscera of the two components situated on diametrically opposite sides of the yolk mass. The intestinal tracts are separate until just before they open at the anus; during the passage from the abdominal cavity to this point they are closely associated side by side as parallel tubes.

The situs of the gut of A is normal, the stomach curving first slightly to the left, then making a wider curve to the right to pass over into the intestine which goes straight caudad to the anus. The liver lies on the right side of the intestinal canal.

The swim bladder, after its origin from the dorsum of the gut well toward the anterior end of the coelom, lies on the left of the canal. The curvatures of the gut of B are normal, as is also the liver, though this organ extends ventrally across the gut farther than normal, displacement being due probably to the pressure of the yolk mass. It sends a small pointed lobule cephalad on the right side of the stomach. The swim bladder of B lies on the right side of the gut immediately after its origin from the dorsal surface and continues in this position to its termination beneath the liver. This abnormal position may also have been due to the pressure of the yolk mass.

No. 2. The vertebral columns are separate to nearly the tip of the tail, the caudal fin is single. The head of A is slightly larger than that of B. The gut of each is normal, showing normal curvatures, and is single to a point slightly anterior to its exit from the body cavity. Here the intestines of the two components fuse. The liver of B is normal in size, shape, and position. That of A is rotated to a more transverse position than usual, otherwise it is normal. The swim bladders are both normal in position.

No. 3 (fig. 1). This specimen shows a typical case of situs inversus in the right twin (component A). The vertebral columns are fused posterior to the pelvic fins, the components are of nearly equal size, externally normal, and so placed that the dorsal fins lie almost in the same plane. The gut passes down the left and right sides of the body walls of components A and B, respectively, to a point about halfway between the pylorus and the exit from the body cavity, where the two fuse into one. Then the common intestine crosses to the ventral side of the coelom and passes back to the anus, where it opens to the outside. The shape of the two stomachs, livers, and swim bladders is normal, but these organs in component A are mirror images of those in component B. The liver of B lies on the right side of the stomach and ventral to the first part of the intestine, with its convex border toward the right; the greater curvature of the stomach is towards the left and is paralleled by the swim bladder. The stomach of A, on the other hand, has its greater

curvature toward the right, and the swim bladder is also on this side. The liver, with its convexity toward the left almost in contact with the corresponding part of the liver of B, is on the left side, its posterior end swinging to the right across the pylorus and the beginning of the intestine.

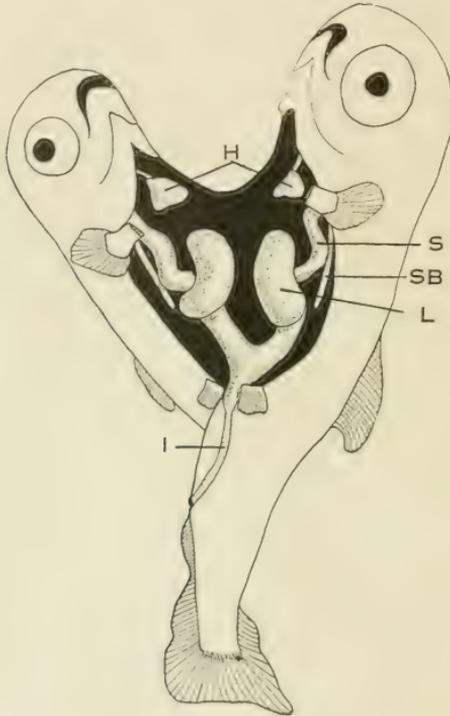


Fig. 1 Ventrolateral view, specimen no. 3. The situs viscerum B is normal, A is reversed. *H*, heart; *L*, liver; *SB*, swim bladder; *S*, stomach; *I*, intestine.

No. 4 (fig. 2 and 3). Situs inversus viscerum of the left twin is clearly shown in this embryo. The two components are of equal size and are placed in almost exact ventral apposition. It was found necessary to cut them apart to show the internal anatomy. The vertebral column of each is single to a point

slightly posterior to the dorsal fin, where the myomeres of the contiguous sides become fused. The adipose fins are separate, though one caudal fin is common to the two components.

The gut of component A is obviously in normal situs, though there is a considerable curvature of the cardiac end of the stomach

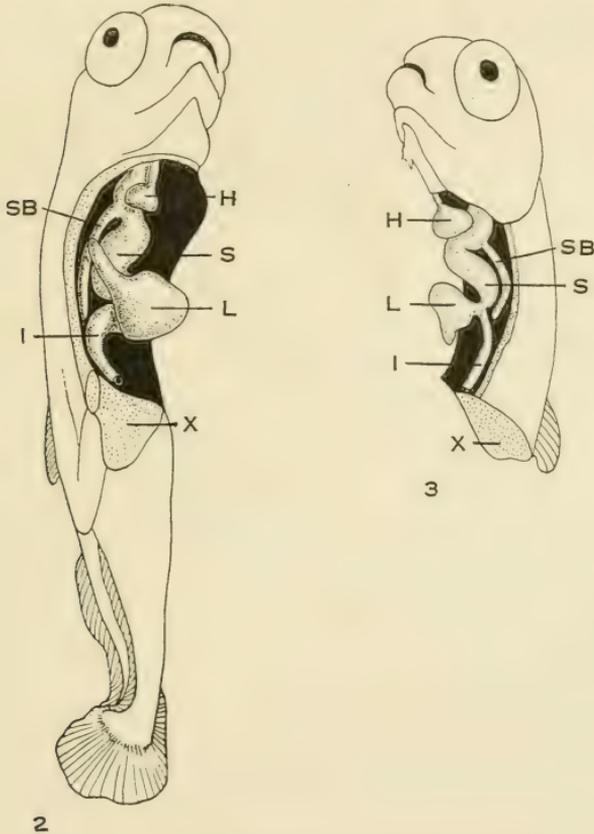


Fig. 2 Component B of specimen no. 4, ventrolateral view. X marks the point of separation from component A (fig. 3), other abbreviations as in fig. 1. This twin shows situs inversus.

Fig. 3 Component A of specimen no. 4, ventrolateral view. Situs is normal. Abbreviations as in figs. 1 and 2.

ventrad and to the right caused by the pressure of the yolk mass upon it. The gut is single until just before its exit from the abdominal cavity, at which point the intestines of the two components fuse and pass to the anus as a single tube. The liver of component A, smaller than normal and somewhat wedge-shaped, occupies a ventral position a little posterior to and to the right of the middle of the common abdominal cavity. It is placed transversely with reference to the gut on the right side of the pylorus. The swim bladder of A is in its normal position at the left of and parallel to the stomach.

The stomach of component B, on entering the abdominal cavity, passes diagonally to the left for a short distance, then, just beyond the origin of the swim bladder, turns sharply to the right; it then curves to the left, gradually at first, then more abruptly beneath the liver, again curving sharply to the right at the pylorus to emerge as the intestine from beneath (dorsal to) the liver immediately caudad to the place where it disappeared. Thence it passes in a direct course to its point of fusion with that of component A. The liver, about the size and shape of that of component A, lies ventral to and covering the pyloric bend, its largest part extending some distance to the left of the gut toward the ventral wall of the common abdominal cavity. It sends an elongate, finger-like process cephalad and to the right over the ventral surface of the cardiac stomach. The swim bladder emerges from the gut on the right side and keeps this position with reference to the stomach and the first part of the intestine to a point near the lower margin of the liver, where it terminates.

No. 5 (figs. 4 and 5). This specimen also shows inversion of situs viscerum in component B. The vertebral columns are separate to a point just posterior to the dorsal fins, where they become fused. The caudal fin is doubled. Component B has an abnormal head, smaller than that of A and possessing no eyes and only a rudimentary mouth. Fusion of the intestines occurs just before exit from the abdominal cavity. The viscera of the autosite (component A) are in normal situs, only slight distortion due to crowding being noted.

The curvatures of the gut of the parasite (component B) are apparently reversed, the stomach first curving to the left and ventrally, then slightly dorsad and to the right. Just posterior to the origin of the swim bladder the greater curvature sweeps

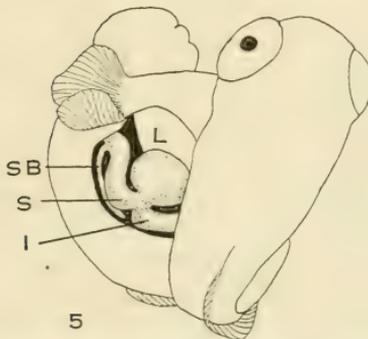
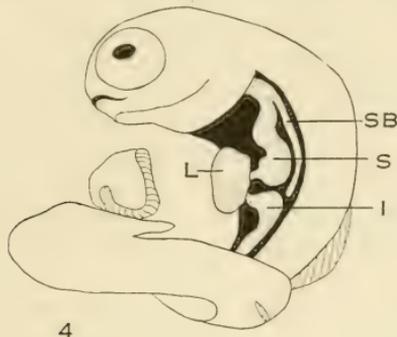


Fig. 4 Left posterolateral view of specimen no. 5, showing arrangement of the viscera in the autosite (component A). The situs viscerum is normal. Abbreviations as in fig. 1.

Fig. 5 Right posterolateral view of the same monster shown in fig. 4, showing the viscera of the parasite (component B). The situs viscerum is reversed. Abbreviations as in fig. 1.

posteriorly to the left and slightly ventrad, turning abruptly dorsad at the pylorus to meet the intestine. The liver is discoid in shape and lies to the left of the pylorus. The swim bladder lies dorsolateral to the stomach on the right side.

No. 6. Fusion of the vertebral columns takes place just posterior to the dorsal fins. Component A shows normal development and situs viscerum, but component B is much smaller, possessing no eyes or nares, and only a rudimentary mouth. Its viscera, however, are well developed, but show such great distortion that it is impossible to locate the primary curvatures with exactness. The liver of A, placed to the right of and ventral to the pylorus, is normal in position though slightly abnormal in shape. Its duct enters the gut posterior to the point where it is joined by the intestine of component B.

The gut of the parasite (B), on appearing in the abdominal cavity, passes transversely across it, then makes a sharp turn caudad and to the left, lying in contact with that of the autosite. At the pylorus it makes a slightly wider turn to the right and very shortly fuses with the pyloric stomach of component A. The liver of B lies to the left of the pylorus close to the dorsal wall, its duct enters the gut before the latter's fusion with that of component A. The swim bladders of both components lie in their normal positions with reference to the stomachs.

No. 7. Fusion of the vertebral columns takes place just posterior to the dorsal fins; more caudad there is a single body and tail. Component A is a very small parasite on component B, the head being only about half the normal size and possessing no eyes, nares, or mouth. The intestinal tracts of the two components are separate throughout, the intestine of the parasite being the more poorly developed and opening dorsal to that of the autosite into the single anus. The situs viscerum of both components is normal, the viscera of the parasite being much distorted anteriorly because of lack of space and considerably underdeveloped posterior to the pylorus.

No. 8. The heads of both components are of the same size and entirely separate to a point just posterior to the gill region. The vertebral columns are separate anterior to the dorsal fin, and both pairs of pectoral fins are present. The gut of each component presents curvatures indicating normal situs, though a considerable degree of distortion is shown, particularly in that of component B. The two fuse near the pyloric ends of the

stomachs. The liver appears as a single bilobed mass lying on (ventral to) the point of fusion of the gut of the two components. The larger lobe is somewhat quadrangular in shape, lying ventral to and to the right of the stomach of component A. The smaller, more anterior lobe of the liver mass extends anteriorly in the midline of the monster, filling the interval between the two stomachs. This anterior lobe apparently represents the liver of component B, and the posterior that of component A. The swim bladders were not found, having probably been destroyed in dissection.

No. 9. Fusion of the vertebral columns takes place just anterior to the dorsal fin (which is doubled). The head of each component is subnormal in size, though the two are approximately equal, that of A showing only a rudimentary lens on the left side, with no external trace of an eye on its right. Fusion of the two alimentary tracts occurs at the pylorus. The curvatures of the gut of both components are normal, but both livers and the swim bladder of B are displaced. The liver of A lies transversely across the ventral side of the first part of the common intestine; that of B, the smaller of the two, lies to the left of and caudad to the greater curvature of the stomach. Its duct, however, can be traced across the ventral surface of the gut to its normal point of communication with it. The swim bladder of A is in its normal position, while that of B appears on the right side of the stomach soon after its origin from the dorsum of the gut.

No. 10. The doubling of the vertebral column in this specimen persists posteriorly to a point midway between the tip of the midbrain and the anterior limit of the dorsal fin. The left and right pectoral fins of A and B, respectively, are coalesced to form an abnormal doubled structure, fused proximally, but distinguishable as two distally. The two heads are normal and of equal size. The cardiac ends of the stomachs of the two components rapidly converge on entering the abdominal cavity so that for the greater part of their length they lie side by side with a very narrow interval between them. Near the pylorus they fuse, and caudad to this point the intestinal tract is common to the two components. The curvatures of the gut indicate a

normal situs. The liver is compound, rather larger than a normal single organ, and is placed transversely across the posterior part of the abdominal cavity. It bears two small, obtuse lobules on the right border, overlapping ventrally the pyloric stomach of component A, and one smaller acute lobule extending cephalad between the stomachs. The swim bladder of component A is not demonstrable, that of B emerges from beneath the stomach to lie in the interval to the left of and paralleling the anterior lobe of the liver mass.

No. 11. The vertebral column is doubled anterior to a point midway between the anterior tip of the head and the dorsal fin. The head of component B is set at almost a right angle from the left side of the gill region of A, and in consequence does not extend so far anteriorly. The left eye of A and the right eye of B are lacking. The pectoral fins of the contiguous sides are fused and are smaller than a normal fin. The gut of component B makes a sharp turn caudad as it appears in the abdominal cavity, extending posteriorly to meet and fuse with that of component A in the pyloric region. From this point on the gut is single. The curvatures of both indicate a normal situs. The compound liver is an abnormally small organ placed slightly ventrad, caudad and to the right of the point of fusion of the digestive canals. The swim bladder of A passes posteriorly in the normal position at the left of the stomach, crossing it dorsally where the stomach makes the final bend before fusing with that of B, and extending still further posteriorly under cover of the liver. The position of the swim bladder of component B was not determined.

No. 12. The vertebral column is single posterior to a point midway between the anterior end of the head and the dorsal fin. The head of component B is smaller than that of A and lacks eyes. Fusion of the gut of the two components takes place at the region of the pyloric stomachs. The situs viscerum B is normal, though the liver is displaced to the left side of the abdominal cavity. The gut of component A presents curvatures of a type almost exactly mirror-imaging those of B. They are, however, not typical and the situs is not clear. The liver,

perhaps in part fused with that of B, shows only as a small mass situated between the two stomachs. The swim bladders were not found.

No. 13. Fusion of the bodies of the two components takes place immediately posterior to the gills. The head of B is normal, that of A is smaller and lacks the left eye. The viscera of the monster show considerable distortion, but the situs of component B is obviously normal. The stomach of component A extends caudad to fuse with that of B in the pyloric region, situs indeterminate. The liver is single and situated on the extreme right side of the abdominal cavity ventral and to the right of the pyloric region. The swim bladders were not visible.

No. 14. Only the head and three cervical segments are doubled, fusion taking place in the vertebral columns a very short distance posterior to the base of the skulls. Both heads are normal and equal in size and development. The stomachs of the two components lie very close together and fuse in the middle of the cardiac regions. The curvatures are apparently normal. The livers are fused and the mass of liver tissue lies ventral to the pyloric bend. The swim bladders are normal in size and position, that of A being hidden from view ventrally by the stomachs and pylorus.

No. 15 (fig. 6). The head of the parasite (component A) is hardly more than a bud from the right side of the anterior trunk region of the autosite, the proper vertebral column being very short. It is almost entirely devoid of recognizable organs, only small rudiments of gills being visible. The guts are fused in the region of the pyloric stomachs. That of the autosite presents normal curvatures, while the parasite shows them slightly reduced and apparently mirror-imaged. The stomach of A passes caudad with very gentle curves first to the left, then a longer one to the right, followed by a sharp turn toward the left to the point of fusion with that of B. A single liver mass (possibly compound) is present, situated on the right side of the abdominal cavity just lateral to the pylorus. It is about the size of a normal single organ. The swim bladders of both components are seen protruding from beneath the right side of the cardiac

stomach of component A, the one from B obviously crowded out of position by yolk, while that of A lies on the right side of the stomach throughout its course.

DISCUSSION

The asymmetry relations in the viscera of the double trout examined in this study bear out in general the view set forth by Morrill ('19) that the correlation between the amount of external doubling and the occurrence of situs inversus viscerum

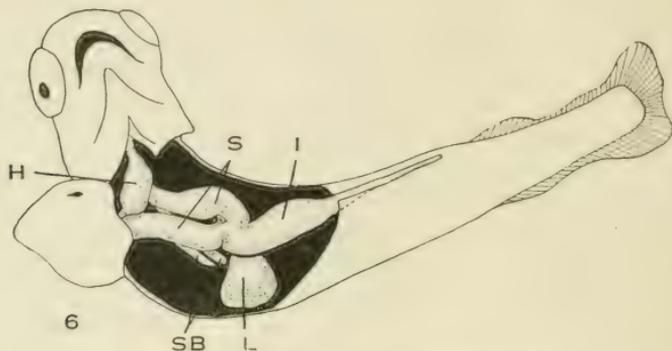


Fig. 6 Ventral view of specimen no. 15. Component A is of the parasite type, B the autosite. Situs B is normal. The gut of A mirror-images that of B. Inversion is not certain; the effect may be due to torsion. Abbreviations as in fig. 1.

is not at all precise. In table 1 the monsters are arranged according to their degree of external doubling. It will be seen that those whose vertebral columns are fused anterior to the dorsal fin and those which are nearly separate show at best only doubtful mirror-imaging. It is further noted, as was also found by Morrill, that not all the animals falling within the limits of doubling apparently most favorable for transposition of the viscera show this phenomenon. Thus there are indications that the reversal of asymmetry is not a necessary consequence of any condition of doubling, but may or may not occur, depending on some other factor capable of operating within these limits.

Column 5 of the table shows the order in which the specimens in this series appear when arranged according to the degree of internal doubling. This indicates a similar condition of correlation to that shown for external doubling, mirror-imaging only occurring when the digestive tracts are fused at some point be-

TABLE 1

Showing trout embryos arranged according to degree of external doubling

NUMBER	SITUS A	SITUS B	REGION OF FUSION VERTEBRAL COLUMN	ORDER OF INTERNAL DOUB- LING	REGION OF FUSION OF GUT
1	Normal	Normal	Base of tail	2	Just anterior to anus
2	Normal	Normal		5	Between pylorus and exit from body cavity
3	Reversed	Normal		6	
4	Normal	Reversed		3	
5	Normal (autosite)	Reversed (parasite)		4	
6	Normal (autosite)	Normal (parasite)	Posterior to dorsal fins	7	Irreg. — pyloric stomach with intestine
7	Normal (parasite)	Normal (autosite)	Anterior to dorsal fins	1	Separate
8	Normal	Normal		12	Pyloric stomach
9	Normal	Normal		9	
10	Normal	Normal	Half way midbrain to dorsal fin	11	At pylorus
11	Normal	Normal	Half way snout to dorsal fin	8	Pyloric stomach
12	Indetermi- nate	Normal		14	
13	Indetermi- nate	Normal	Just posterior to gills	10	At pylorus
14	Normal	Normal	Ca. 3rd cervical segment	15	Midcardiac stom- ach
15	Reversed ? (parasite)	Normal (autosite)	Near base of skull	13	Pyloric stomach

tween the pylorus and the exit of the intestine from the abdominal cavity. The table clearly shows that there is no accurate correspondence between the degree of external and internal doubling. Spemann and Falkenberg ('19) find that situs inversus occurs in separate twins produced by constriction of the embryo at blastula or gastrula stage, and in the light of this it cannot be

said that mirror-imaging is in any way dependent on the degree of doubling, external or internal.

It is not in point to discuss in detail at this time the theories of the causes of situs inversus, and the reader is referred to Morrill ('19), pp. 275-81, for a concise review of the recent literature. Pressler ('11), working with Spemann's material, found situs inversus in larvae of *Rana esculenta* and *Bombinator*, which had had a portion of the medullary plate and the subjacent endoderm turned end for end in the neurula stage. Spemann ('18), working also with *Bombinator*, has shown that situs inversus can be obtained by inversion of a small bit of ectoderm and endoderm in the region of the future medullary plate at the end of gastrulation, but inversion of the ectoderm alone at the beginning of gastrulation has no effect. Spemann and Falkenberg ('19) found in the twins and double monsters of *Triton* produced by constricting the developing embryo at the gastrula, blastula, or even earlier stages, that approximately half of the right twins (corresponding to component A) showed situs inversus, the others being normal or of indeterminate situs. Spemann, in discussing the theories of the causal factors in situs inversus, considers a fundamental inherent bilateral asymmetry in the egg. He suggests as a possible interpretation of the occurrence of mirror-imaging in the right twin after a cut in the midline of the embryo, that the primary asymmetry existing in the right half of the gut anlage may be reversed in some such manner as is that of certain asymmetrical crystals after injury. He considers that this explanation, which was put forward by Přizibram to account for reversal of asymmetry in a limb regenerated from a proximally directed wound surface, may be at least partially applicable to the phenomena which result in the establishment of situs inversus viscerum in the right twin. The possibility of reversing the microstructure of crystals by cutting seems to him highly suggestive in accounting for mirror-imaging in the gut, as inversion of the microstructure of the anlage of one twin would, in the course of subsequent normal development on this basis, produce situs inversus, just as normal development from a normal (not inverted) micro-

structure would result in normal situs. However, in view of the fact that constriction of an embryo of practically any age up to the completion of gastrulation may result in situs inversus viscerum of one of the twins so produced, Spemann considers it more probable that asymmetry reversal in the viscera is brought about as a direct effect of the injury rather than an inversion of the fundamental microstructure. Structural deficiencies are observed to preponderate on the operated side in such experimental animals and the bodies are often definitely bent toward this side. This shows there is a marked effect from the wound, and this influence may be sufficient to transpose the relations of the viscera without any molecular rearrangement in the cellular make-up. The exact manner in which these fundamental changes are brought about remains for future investigations to determine. Spemann suggests that breeding animals with situs inversus may help in clearing up this confusion.

Aside from the fundamental problem of the causes of symmetry reversal, another point deserves mention. Morrill ('19) states that it is component A which shows the mirror-imaging, if inversion is present, component B always being normal. Spemann, in the work mentioned above, describes an exceptional case showing mirror-imaging of the heart of the left twin; the situs of the gut of this twin was not clear. There was also mirror-imaging in the gut and heart of the right twin of this pair. He states that situs inversus almost never occurs in the left twin. In my cases no. 4 and no. 5 we have two more examples of inversion in the left twin (component B). Spemann, in explaining the inversion of situs cordis which he found in this left twin, states that it is probably due to an influence other than that of the operation, affecting the heart anlage from the outer side. In support of this view he cites cases of his own which show defects of the limbs, gills, etc., on the side of the body away from the cut, which have resulted from stimuli not connected with his operative procedure. He also brings up the experiments of Dareste ('77), who produced situs inversus ('l'hétérotaxie') in chicks by the application of excessive heat to the left side of the developing embryo, as corroborative of this point of view.

This work was later repeated by Warynski and Fol ('84) with similar results. It seems improbable that the extensive conditions of inversion found in the two double monsters no. 4 and no. 5 of this study could be due to the effect of some adverse external influence applied in just the right region and at precisely the right moment. As is shown by figs. 2, 3, 4 and 5, there are no unilateral structural abnormalities in either of these cases to be related to retarded or accelerated growth. Both components of no. 4 are externally normal, and though component B of no. 5 is of the parasite type, it is at least bilaterally symmetrical. Again, it is stated by Morrill that in his specimens of the auto-site-parasite type, where one component is considerably larger and better developed than the other, mirror-imaging never was found in the parasite, no matter on which side of the monster it was situated. Here it is necessary to call attention to specimen no. 5 (and possibly no. 15), in which it is the parasite which shows the inversion. Interpreting *situs inversus* according to the theory set forth by Spemann, it is difficult to see why the relative size of the twins should influence the occurrence of mirror-imaging. Indeed, if there were an effect, one might expect that the parasite, showing the poorer development, would more easily be affected than the autosite.

At present the ultimate cause of *situs inversus* is still hypothetical: the theories which have been advanced for its explanation are suggestive, but not conclusive. There are indications of a general correlation between the degree of doubling and the occurrence of mirror-imaging in the viscera, but since separate twins may also show *situs inversus*, the significance of any such correlation is very doubtful. *Situs inversus* may occur in either twin, though the large predominance of rights over lefts in this respect indicates that for some reason the two sides are not equally susceptible. Twins of the parasite type may show *situs inversus*. It is evident that more data and further experiments along these lines are necessary before a true solution of the problem can be reached.

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Resumen por la autora, Evelyn Holt.

Un proceso peculiar en el piso diencefálico del feto de ternera.

En el piso diencefálico del feto de ternera existe un proceso peculiar que aparece por primera vez en el estado de 48 mm. existiendo en todos los estados ulteriores. Este proceso se origina como una masa irregular que parte del límite posterior del quiasma en la línea media ventral, extendiéndose hacia abajo y posteriormente en el espacio subaracnoideo, para terminar de un modo difuso en el mesenquima de la región de la parte bucal de la hipófisis. El proceso mencionado no presenta nunca conexión alguna con otras estructuras y parece ser de naturaleza neurógica. Mientras que existe constantemente en el embrión de ternera, no ha podido hallarse en el cerdo, carnero o en el hombre.

Translation by José F. Nonidez
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A PECULIAR PROCESS OF THE DIENCEPHALIC FLOOR IN THE FETAL CALF

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ONE FIGURE

From the diencephalon of the developing calf embryo is a curious ventral outflow of nervous tissue which was pointed out to me by Doctor Kingsbury and which hitherto has not been described. This structure does not appear in the early stages, but from the time of its appearance, in a 40-mm. embryo, was found present in all the specimens examined.

For this study series of older calf embryos were used. Specimens showing the head region cut in the sagittal plane included the following lengths: 30, 48, 58, 62, 80, 82, 94, 95, 110, 130, 167, 190, and 310 mm. In addition embryos of 33, 39, 40, and 50 mm. cut in the transverse plane were used for purposes of comparison. These embryos, the property of the Department of Histology and Embryology of the Cornell University Medical College at Ithaca, are cut and mounted serially, and stained with hematoxylin and eosin, hematoxylin and orange G, or hydrochloric acid carmine, with or without Lyons blue as a counterstain. All except the youngest specimens were decalcified before cutting.

The structure to be described first appears as a definite outflow in the 48-mm. stage, although there are indications of it at 40 mm. From the time of its appearance it is constant, becoming increasingly prominent in the older stages, and having certain characteristic relations throughout. These are shown in the accompanying figure, an untouched photograph of the hypophyseal region of a 110-mm. calf embryo. The photograph which represents a sagittal section near the midline is reproduced at a magnification of ten diameters. Arising as an irregular mass

from the posterior limit of the chiasma in the midventral line, this process of neural tissue extends downward and backward to terminate diffusely in the mesenchyme near the pars buccalis of the hypophysis. In all the specimens the process faded out in an indefinite brush-like manner, the ragged ends becoming lost in the mesenchyme, and never showing a connection with any other structure. It is interesting to note that even before



Fig. 1 Sagittal section near midline of hypophysial region of 110-mm. calf embryo. $\times 13\frac{1}{2}$. *p*, pons; *c*, optic chiasma; *in*, infundibular process (pars nervosa of hypophysis); *b*, pars buccalis of hypophysis; *sp*, basilar plate (sphenoid); *x*, process herein described; *a*, developing arachnoid and dura (inner layer); *1*, subarachnoid space; *2*, mesenchyme of sella turcica.

the appearance of this structure as a definite outflow its point of origin is marked by the entrance of one or more small blood vessels into the neural tube. This fact may or may not have significance, but is rather noticeable because of its constancy. This outgrowth shows no signs of axis cylinders, is in no way connected with a ganglion, and appears to be neuroglial in character. Little, however, can be said concerning its actual struc-

ture, as it was not studied on material stained especially for nervous tissue.

Any description of the process must include a brief mention of the developing meninges and the hypophysis, to which structures it bears an intimate relation. Weed ('15) described the developing brain membranes of the pig, and his findings apply to the calf also. A mesenchymal condensation directly around the neural tube represents pia, while outside this the mesenchyme takes on the character of a loose meshwork, the spaces enlarging and the arachnoid trabeculae appearing as irregular condensations. Beyond the arachnoid trabeculae is a condensation which represents the arachnoid membrane and the inner layer of the dura. In the pig (Weed, '15) the line of separation between these two appears at 50 mm. In the calf the separation process is evident by 48 mm., but progresses rather slowly. The processes of mesenchymal arrangement and condensation which result in the formation of the meninges are well advanced before the first appearance of the outgrowth, and locating the outgrowth in terms of the membranes we may say that it is always confined to the subarachnoid area, never extending beyond the condensation which represents arachnoid and dura. As is well known, these membranes form a collar, the diaphragma sellae, about the neck of the hypophysis. This collar is pierced by the infundibular stalk and the pars tuberalis. The neural process confined to the subarachnoid space and directed toward the hypophysis suggests the possibility of a close, perhaps a functional, relation between the outflow and the gland. The best indication of this would be an actual contact between the two structures. Such a contact could never be observed. At its origin, however, the outflow bears a definite spatial relation to the pars tuberalis of the hypophysis. This tongue-like process (to use Herring's term) extending forward over the surface of the tuber cinereum ('postchiasmatic eminence'—Tilney, '15) stops immediately before it reaches the outgrowth, being separated from and bound to that structure by its own basement membrane, which is continuous with the pia, but which appears somewhat thicker and more compact than the typical developing

pia. The nasal horns of the tuberal processes (Atwell, '18) have fused across the midline before the appearance of the outflow.

Curiously enough, although a number of investigators (Herring, '08; Trautmann, '09; Wulzen, '14) have studied the hypophysis and hypophyseal region in the calf, no one has described this structure. Nor has it, so far as could be determined, been described in any other form. It is, as has been said, constant in all the calf embryos examined from the 40-mm. stage to and including a specimen 310 mm. in length. No embryos older than this were examined. The process is not mentioned by Tilney ('15) in his study of the comparative morphology of the diencephalic floor, and could not be found in pig, sheep, or human embryos. The pig embryos belonging to the Department of Histology and Embryology include lengths up to 54 mm., while the sheep embryos, kindly lent by Professor Gage, include lengths up to 44.5 mm. Because of the possibility that the structure might appear in older embryos than those examined, the diencephalic region of a 90-mm. lamb was studied. No sign of the nervous process was present. This nervous process is present in the calf, absent in the pig, sheep, and human, and undetermined in other forms.

While no theory is offered to explain this process, it is believed that the explanation will be found, not in the study of it alone, but in its relation to surrounding structures, especially the hypophysis cerebri, the mesenchyme, and the developing meninges. It may represent a portion of the brain wall which became fixed—possibly by a developing blood vessel—and was subsequently drawn out into an irregular process by the growth of surrounding parts, or it may indicate some primitive, little known, condition of development. Why it should exist in the calf, and apparently in the calf only, remains a puzzle. It seems to have no function, no homologue, no obvious reason for existing, and yet because it is constant in this form there must be some underlying cause, some point wherein the development of the diencephalic or hypophyseal region in the calf differs from the condition in other forms. This can be determined

only with a fuller understanding of the development of this region.

In conclusion, I should like to thank Doctor Kingsbury for the help which he has so generously given.

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Resumen por la autora, Evelyn Holt.

Ausencia de la parte bucal de la hipófisis en un embrión de cerdo de 40 mm.

El objeto del presente trabajo es la descripción de un embrión de cerdo de 40 mm., el cual aunque parecía normal en todos los demás rasgos, no poseía la parte bucal de la hipófisis. Mientras que dicha parte bucal falta, la parte neural está bien desarrollada, estando reunida con el cerebro, y presentando posición, tamaño y estructura normales. Está rodeada por una condensación de mesenquima que es continua con la pía y se parece a una membrana basal, excepto cerca del ápice del proceso, donde la diferencia entre los tejidos epitelial y neural se pierde por completo. La tiroides, suprarrenales y gonadas aparecen normales cuando se las compara con embriones de cerdo de 38 y 42 mm., respectivamente. Como no se ha descrito ningún caso semejante, esta anomalía debe considerarse como rara. Tiende a rechazar la teoría que atribuye el desarrollo del proceso infundibular a la presión ejercida por la bolsa de Rathke sobre la vesícula cerebral anterior.

Translation by José F. Nonidez
Cornell Medical College, New York

ABSENCE OF THE PARS BUCCALIS OF THE HYPOPHYSIS IN A 40-MM. PIG

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TWO FIGURES

Belonging to the Department of Histology and Embryology at the Cornell University Medical College in Ithaca is a 40-mm. pig embryo, remarkable for the fact that, while the infundibulum is well developed, the oral portion of the hypophysis is wholly absent; in other respects the embryo appears normal. Because no similar case has been recorded and because this abnormality may help to make clear the normal development of the hypophysis, a brief description of the specimen is here given.

The embryo (series 148) is well preserved, cut transversely in sections 10 μ thick, and stained with hematoxylin and eosin. For purposes of comparison, there were used two pig embryos of 38 and 42 mm., respectively. These were also cut in the transverse plane. Photographs at a magnification of twenty-two diameters were made of the hypophyseal region of the 40 and 42-mm. pigs, and are here reproduced, side by side, as figures 1 and 1a, 2 and 2a, because it is believed that the untouched photographs present the true condition more accurately than line drawings or verbal descriptions. Owing to the inevitable slight difference in the plane of section, the levels do not correspond exactly.

In the 40-mm. pig the pars buccalis of the hypophysis is wholly absent, there being no trace of it in the usual position about the pars nervosa—where at this stage it should be a conspicuous feature—or in the roof of the pharynx, or along the route of development, where a craniopharyngeal canal sometimes exists. The pars neuralis, on the other hand, is well developed, connected with the brain, and normal in position, extent, and



1



1a



2



2a

structure. It is tubular, extends downwards and backwards from the diencephalic floor, and is made up of two zones, a medulla consisting of several layers of closely packed cells, and a cortex made up of fibers and cells in looser arrangement. This point, the normal appearance of the pars nervosa, is of special interest in connection with the work of Smith ('20), who removed the anlage of the epithelial hypophysis (pars buccalis) from frog larvae of 3.5 to 4 mm. in length.¹ On the basis of his work, Smith says (p. 78): "It thus seems clear that in the absence of a nearly normal epithelial component, the neural hypophysis can not undergo its normal development nor attain its typical size or shape." He found the pars nervosa in the operated tadpoles to be smaller than normal, asymmetrical in

Fig. 1 Section of the hypophyseal region of a 40-mm. pig embryo, showing complete absence of the pars buccalis.

Fig. 1-a Comparable section of a (normal) 42-mm. pig embryo.

In the mesenchyme of the sella turcica appears the pars nervosa, alone in figure 1, nearly surrounded by pars buccalis in figure 1-a. On either side of the hypophysis is the corresponding internal carotid artery. Anteriorly (above) the optic chiasma may be seen, while posteriorly (below) is a part of the cartilage of the sella turcica.

Fig. 2 Section of 40-mm. pig showing infundibular stalk connected with the brain.

Fig. 2-a Comparable section of 42-mm. pig.

From before backward the following parts of the brain appear, optic chiasma, postchiasmatic eminence (medial eminence of tuber cinereum), pars nervosa of hypophysis, and, behind the sella turcica, pons. The fact that in figure 2 the chiasma and postchiasmatic eminence appear continuous, while in figure 2-a they are separated by mesenchyme, is due to a slight difference in the plane of section. In figure 2 the pars buccalis is lacking and the pars nervosa is directly related to the mesenchyme, while in figure 2-a the pars buccalis is present appearing on either side of the infundibular process and extending forward (pars tuberalis) over the surface of the tuber cinereum. The internal carotid arteries appear as before.

¹ Smith classified the results of the operation as, 1) disturbances of pigmentation; 2) changes in the rate of growth, and, 3) endocrine disturbances. The first of these does not apply to the pig and will not be considered here. The second is difficult to determine from one specimen, especially as we have not the litter mates to use as a basis of comparison. In point of differentiation, however, the embryo is comparable to other pigs of the same length. The third point, endocrine disturbances apart from the pars nervosa of the hypophysis, will be considered a little later.

form, and atypical in position. As the oral component was removed before the formation of the primitive infundibulum and without injury to the neural tube, these changes were not considered traumatic, due to the operation. They might be explained as due to one of two factors or to these two acting conjointly. They might be an expression of a general endocrine disturbance due to hormone deficiency, atrophic or non-developmental changes comparable with the marked peculiarities noted by Smith (pp. 83 to 97) in the thyroid and adrenals; or they might be due to the lack of a mechanical stimulus normally derived from the pars buccalis. While he does not deny the possibility of the former factor, Smith states quite plainly (p. 83) that "the neural lobe and pituitary floor are dependent upon the association with the epithelial hypophysis for their full development." The pars nervosa of this pig embryo, being normal in position, size, and shape, does not support the theory that the association of a normal pars buccalis is necessary for the development of the infundibulum. It is probable, however, that the presence of the whole gland would be necessary for the complete development and normal functioning of the pars nervosa. Here we encounter a difficulty, as the exact functional processes of the gland and the relation of the two parts are little understood. That the relation is a close one seems evident on histological grounds. Atwell ('18, pp. 303, 304, fig. 21) states that in sixteen-day rabbit embryos there are definite contacts between the two portions of the hypophysis, these contacts indicating outgrowth of one or the other part. These may also be observed in pig and calf embryos. Herring ('08 a, p. 149) says that in adult cats epithelial cells may be demonstrated in the infundibular process, these cells having migrated from the pars buccalis. In that these contacts and ingrowths obviously cannot exist in the absence of the pars buccalis, the pars nervosa cannot reach full development alone; but that in the absence of the pars buccalis the infundibular stalk and process not only may appear, but may appear normal, in the mammal at least, is demonstrated by this embryo.

The infundibular process is surrounded by a mesenchymal condensation which is continuous with the pia and resembles a basement membrane except near the tip of the process where the line of demarcation between the two tissues becomes lost and there seems to be a confluence of neural and mesenchymal tissue. Here the nervous tissue appears to end in an irregular scalloped manner, while the mesenchyme filling in the irregularities (fig. 2) has the appearance of actually invading the nervous process. This is probably normal, as the same thing was observed in sagittal sections of 35- and 54-mm. pigs. Atwell ('18) states that in the rabbit portions of the original basement membrane may be seen between the cortical and medullary zones of the infundibulum. It was perhaps this appearance which led Müller and Mihalkovics ('75) to believe that mesenchymal cells replace the proper nervous tissue of the infundibulum, converting it into a connective-tissue appendage of the brain. Herring ('08) showed that connective tissue is never present in the infundibulum to a considerable extent, the fibers which were described as connective tissue being ependymal or neuroglial in character. The condensation which represents the arachnoid and inner layer of the dura appears to surround the neck of the infundibulum as it would were the whole gland present.

Upon careful examination, the development of other parts appeared normal, and that the embryo was alive and growing is proved by the presence of mitotic figures. The mesenchyme of the sella turcica shows evidences of erythrocyte formation and does not differ appreciably from the mesenchyme of this region in a normal embryo. The notochord ends, as in the case of the 38- and 42-mm. pigs, in the cartilaginous sphenoid. How it ended in an earlier stage when, in the pig, it would normally be attached to the wall of Rathke's pocket, we have, unfortunately, no means of determining.

Because of the emphasis placed by various workers on the relation between the hypophysis and the other ductless glands, the thyroid, gonads, and suprarenals of the three pigs were compared. The results of this comparison were negative. In

the 40-mm. pig the thyroid was slightly larger than in either of the others, but this difference did not seem sufficient to be connected in any way with the non-development of the hypophysis, especially as the gland was a trifle larger in the 38- than in the 42-mm. pig. The structure of the gland was similar in all cases, a well-vascularized cord- or platework of cuboidal cells with deeply staining nuclei. Colloid was not yet present. The ovaries of the 40- and 42-mm. pigs were similar in state of development. The 38-mm. pig was a male. The suprarenals seemed to be entirely normal. These observations differ from the findings of Smith ('20), but the difference may well be due to the fact that the pig develops in utero and receives pituitary secretion from the maternal organism. At any rate, this embryo offers no evidence of a primary relation between the endocrine organs.

While there are numerous descriptions of hypophyseal tumors and accessory glands, such as the pharyngeal hypophysis situated in the roof of the pharynx (Cushing, '12, and Schwalbe, '09), it has been impossible to find the record of a single case of absence of either lobe. As the *pars buccalis* is considered essential to life, an animal presumably could not live after birth with this portion of the gland congenitally absent. In this connection perhaps it should be noted that Smith's hypophysectomized tadpoles were never able to complete metamorphosis. As the literature contains no account of such an abnormality, whether in a fetus or in an animal after birth, it must be assumed that the defect is rare. Because neither part of the gland has been described as existing without the other, most workers have assumed the relation between neural and buccal parts to be fundamental.

Although there are several theories to account for the development and relations of the two parts of the gland, no one affords adequate explanation of this anomaly. His, Dursy and Müller, believed that the notochord exercised a mechanical influence in drawing out the infundibulum. Mihalkovics ('75), however, disproved this theory, and again Woerdemann ('14) pointed out that what the earlier workers had described as contacts

were not true contacts—that is, while the two parts may be close together they are always separated by a slight amount of connective tissue. Others, notably Reichert and His, noting the contact between the notochord and Rathke's pocket, held that the notochord served to draw out the oral portion of the hypophysis. Mihalkovics ('75), Woerdemann ('14), and Atwell ('18) disproved this theory, basing their conclusions on the following observations: The contact does not exist until after Rathke's pocket is formed; that is, the 'cause' follows the 'effect.' The contact is by no means constant; in some forms it appears at the superior pole of the pocket, in others at the inferior, while in the rabbit it is variable, present in some but not in all specimens.

Mihalkovics, observing that the oral evagination is the first to form and that it is closely related to the neural tube, attributed the formation of the infundibulum to the pressure exerted by Rathke's pocket. Or, as Herring ('08 a, p. 163), who adopted this theory, said, "The wall of the sac presses upon the base of the anterior brain vesicle, giving rise at its upper extremity to a fold which becomes the primitive infundibulum." This theory fails to explain the presence of the infundibulum in the absence of the pars buccalis, unless we assume that Rathke's pocket appeared, pressed upon the base of the brain to start an evagination, and then involuted quickly and completely. We have no basis for this assumption, and it would leave us with three problems instead of one—What caused the developing pocket to disappear? Why did not this factor cause some other abnormality? Why is there no trace of an oral evagination?

Wiley ('94, p. 287) believed the relation between neural and oral epithelia to be incidental, due perhaps to a tendency of contiguous embryonic tissues to fuse together. This seems improbable, because the relation between the two parts is close and constant, being marked from the earliest stages.

Minot ('92, pp. 573, 574) advanced another theory: "The ectoderm of the mouth over the hypophyseal area lies against and is apparently intimately soldered to the ectoderm of the brain, a point which has been generally overlooked, but which

seems to me of great importance. . . . It is probable that the cementing together over the hypophyseal area of the buccal and cerebral ectoderm is the mechanical condition causing the formation of the two diverticula." On the basis of this theory, we might explain the absence of the pars buccalis in the following manner: the neural and oral epithelia came together as usual (before the formation of the hypophyseal angle), then the growth of the embryo was disturbed so that Rathke's pocket failed to appear, the disturbance subsided, and the infundibulum, normally appearing somewhat later, developed as usual.

Perhaps it would be better to consider the development of the hypophysis in connection with the development of the rest of the head, paying particular attention to the mesoderm and the head bend. The cementing together of neural and oral epithelia without the intervention of mesenchyme is perhaps important as affording a fixed point. Then, Rathke's pocket would be due not to a simple evagination of oral ectoderm, but to the fact that at one point two layers of epithelia are held together, while at all other points they are separated by mesenchyme, the degree of separation increasing with the proliferation of the mesenchyme cells and the growth of the surface ectoderm. If at an early stage mesenchyme cells appeared between the oral epithelium and the wall of the brain, there would be no fixed point and consequently no Rathke's pocket. If this were so, the presence of the infundibulum would be determined by the growth of the neural tube in its relation to the surrounding mesoderm. The anomaly here described, while it renders improbable Mihalkovics' theory as to the mechanics of the development of the hypophysis, proves no other theory. It stands as an interesting and rare anomaly, an occurrence which must be explained by anyone who attempts to account for the development and relations of the two parts of the hypophysis cerebri.

To Doctor Kingsbury, who suggested this description and placed at my disposal the departmental material making it possible, I wish to express my thanks for his very generous help.

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ABSTRACT

THE VASCULARIZATION OF THE MEMBRANOUS LABYRINTH OF THE GUINEA-PIG, THE RABBIT, AND THE CAT.¹ By Denjiro Nabeya, M.D., Los Angeles, California. From the Anatomical Laboratory of the University of Chicago. Abstracted by George E. Shambaugh.

This is a careful piece of anatomical research undertaken on the suggestion of Doctor Shambaugh, who had previously worked out the blood supply of the labyrinth in the domestic pig, the sheep, and the calf. These investigations by Nabeya constitute a valuable addition to the study of comparative anatomy.

The methods employed were the celloidin casts as used by Eichler, Siebenmann and Shambaugh, supplemented by modification of the Spalteholz method of rendering anatomical preparations transparent.

In the arterial system for each of the three animals studied, the same general scheme was observed as that found by Siebenmann for the human, Asai for the dog and the rat, and Shambaugh for the pig, the sheep, and the calf. A single artery supplies the entire labyrinth, entering through the meatus acusticus internus. The first branch is a vessel which follows the ramus utriculus ampullaris and supplies the utricle and the anterior crurae of the superior and the horizontal canals. The opposite crurae of these two canals, the crus commune and the posterior canal are all supplied from a second branch. The first vessel is usually termed the anterior vestibular artery and the second the posterior vestibular artery. The relation of the latter to the vessel supplying the basal coil of the cochlea differs in different animals. Nabeya found in the guinea-pig that two parallel arteries supplied each crus of the semicircular canals. This relation has not been observed in other animals studied. Special interest lies in his findings regarding the venous system. Siebenmann had found three veins draining the human labyrinth. One vein left the labyrinth along the aquaeductus cochleae, a second along the aquaeductus vestibuli, and the third along the meatus acusticus internus. Shambaugh found in the pig all the veins of the labyrinth united into one vessel which left along the aquaeductus cochleae. This same condition was found in the sheep, while in the calf as an anatomical variation a small vein was occasionally found leaving along the aquaeductus vestibuli. In none of the animals

¹The original paper was so extended and had so many colored illustrations that it was decided by Professor Bensley not to attempt publication in American journals. Doctor Nabeya will publish the main paper in Japan, but since the text, in the Japanese language, will not be available to most of those interested in the results, the present abstract is printed here.

studied did he find a vein in the meatus acusticus internus which drained vessels from the labyrinth. Later Asai, working in the laboratory of Siebenmann, seemed to corroborate in the dog and the rat the veins described by Siebenmann for the human. Nabeya in his studies made a special effort to determine this question of the veins' leaving the labyrinth. His results are in accord with the observation of Shambaugh, that in both the guinea-pig and the cat all the veins draining the labyrinth are collected into a single trunk which leaves along the aquaeductus cochleae. In the rabbit, in addition to this vein, he found a vein draining the vestibule and the semicircular canals which leaves along the aquaeductus vestibuli. Only in the cat was he able to make out a vein in the meatus internus, which, however, he does not believe is a vessel belonging to the internal ear. The special method employed in his research left no possible chance for the destruction of these vessels, as Siebenmann and Asai have suspected in their criticism of the work of Shambaugh.

As a rule, there are two veins which drain the vestibule and the semicircular canals, the anterior vestibular vein draining the region supplied by the anterior vestibular artery and the posterior vestibular vein draining the region supplied by the posterior vestibular artery. Shambaugh had found in the sheep that the anterior vestibular vein was wanting and that all the veins from the vestibule and semicircular canals were collected by one trunk, the posterior vestibular vein. Nabeya found in the cat a similar arrangement by which a single vein drains the utricle and all of the semi-circular canals.

No blood vessels, as a rule, are found either in Reissner's membrane or connecting the vessels of the lamina spiralis with those of the spiral ligament, the zona pectinata of the membrana basilaris remaining free from vessels. Shambaugh had found in the pig that the membrane of Reissner was without blood vessels, but in the zona pectinata he found an occasional vessel connecting the spiral vessel under the tunnel of Corti with the vessels in the spiral ligament. In the sheep and the calf he found vessels in both the membrane of Reissner and in the zona pectinati. Nabeya found in the guinea-pig no vessels in Reissner's membrane or the zona pectinata. In the rabbit, however, he found vessels in both of these structures.

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THE ENDOTHELIAL PROBLEM¹

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FOREWORD

I think we now all recognize the general principle that the endothelium of blood-vessels may arise in loco in the body-axis of the embryo, a view which is opposed to the well-known angioblast or ingrowth theory of His. My speech this evening will be merely a survey of the direction taken by a long line of investigations, made chiefly by American anatomists, which has finally led up to the establishment and recognition of this fact. No further claim is made for the principle of a local origin of lymphatic endothelium from mesenchyme, beyond what has previously been stated in print. It will be necessary, however, to refer to certain investigations on the lymphatic system, as it is largely through them that we have been guided in arriving at the facts.

This seems an auspicious occasion to review this subject in general, not only as a tribute to the work of a large group of American anatomists, but also for the reason that such a review may serve as a guide to the younger generation of anatomists who may care to continue this work.

Since endothelium is the essential tissue of the vascular system, the question has naturally arisen as to the manner in which this endothelium makes its appearance in the course of ontogeny; also, where and when it arises in the embryo and how the endothelial-lined channels of the vascular system are established. Two opposing theories have been advanced by European anatomists in answer to these questions: A. The angioblast theory of His; B. The local-origin theory.

¹ President's address; read before the American Association of Anatomists, at their annual dinner, held in Philadelphia, on March 25, 1921.

A. THE ANGIOBLAST THEORY

It has been observed in meroblastic ova, like those of the domestic fowl, that blood-vessels and blood-cells seem to make their first appearance on the yolk-sac and then appear at a somewhat later stage of development in the body-axis of the embryo. The early appearance of the blood-vessels on the yolk-sac led His to infer that certain cells on the yolk-sac, which have been described by some as derivatives of entoderm and by others as derivatives of mesoderm, undergo an early differentiation to form a specialized tissue from which the endothelium of the yolk-sac vessels and the blood-cells are exclusively derived.

This supposedly precociously developed vascular tissue formed on the yolk-sac has been termed the *angioblast* by His, who regarded it as forming a local unit vascular anlage from which, in addition to the endothelium of the blood-vessels on the yolk-sac, that which appears within the body of the embryo is also directly derived. According to the angioblast theory, the yolk-sac angioblast grows into the embryonic axis from the yolk-sac in a continuous and uninterrupted manner, thereby supplying to the embryo all of the material which subsequently gives rise to the endothelium of the entire intraembryonic vascular system. This theory that the yolk-sac angioblast forms the unit vascular anlage of the entire vascular system precludes the possibility that the intraembryonic endothelium arises from any tissue in the embryonic axis other than the invading angioblast. The gradual and progressive manner in which the angioblast is supposed to grow into the embryonic axis also necessarily implies that no discontinuity between the angioblast on the yolk-sac and any portion of that which has invaded the embryonic axis can possibly exist at any time or place. The angioblast is therefore regarded as being a highly specialized tissue, early differentiated during embryonic development, and at first confined to a localized area, the yolk-sac. That all intraembryonic endothelium is derived from this local unit vascular anlage by a process of continuous growth, and that this fact marks the specificity of the angioblast and its derivatives, is one of the primary and fundamental axioms of the

angioblast theory. Another essential feature of the angioblast theory is that all intraembryonic endothelium invariably arises from some preexisting endothelium. If it did not arise in this manner, it would have a local origin from some tissue other than endothelium.

The implied specificity of yolk-sac angioblast, the origin from it of intraembryonic endothelium by a process of continuous growth, and the necessity thereby implied that all intraembryonic endothelium arises from some preexisting endothelium, constitute the logical claims of the adherents of the angioblast theory.

B. THE LOCAL-ORIGIN THEORY²

The view opposed to the angioblast theory is that of local origin. According to this view, mesenchyme may, in practically any region of the body, transform into vascular tissue. The cells which bound an intraembryonic blood-vessel are not in direct lineage with those which line the early vessels on the yolk-sac; they have not come into being as an ingrowth from the early yolk-sac vessels or angioblast; they have not necessarily come from preexisting endothelial cells, though some of them may have had such an origin, inasmuch as local origin does not preclude the possibility of growth during or following the process of local vascular formation. Addition to endothelium may take place, 1) by proliferation of endothelial cells already formed; 2) by addition of single mesenchyme cells; 3) by addition of solid cell aggregates; 4) by addition of already formed endothelial cavities, the lining cells of which have differentiated locally, in and from the mesenchyme, and, 5) by the active migration and alignment of single mesenchyme cells to form vascular cavities. The local-origin theory holds that blood-cells are not necessarily descended from a primitive yolk-sac angioblast, but that mesenchyme within the embryonic body is capable of giving rise to blood-cells. Advocates of the local-

² With only slight modifications, this account of the local-origin theory has been taken bodily from Reagan's paper, in volume 21 of *The American Journal of Anatomy*.

origin theory do not believe that the vascular anlagen are necessarily differentiated at a very early stage of development, as claimed by His, or collectively and at one time, as stated by Minot; advocates of the mesenchymal theory recognize that there are certain regions in which a precocious production of vascular tissues takes place, but they claim that such regions are not the only regions in which such tissues are formed. Advocates of local-origin theory recognize various intraembryonic regions in which there is a first-hand production of vascular tissues, even relatively late in ontogeny, quite independent of such processes in the yolk-sac. The angioblast theory regards endothelium as a tissue of high specialization quite foreign in nature to mesenchyme and quite removed from it genetically. The local-origin theory claims that mesenchyme can transform into endothelium and that endothelium can transform into mesenchyme.

The theory of local mesenchymal origin of endothelium dates back to the work of Reichert ('62) and Goette ('74). Within more recent times, Rückert and Mollier ('06) and their students have chiefly constituted the European School which maintains that endothelium develops *in situ*. Von Felix ('97), Maximow ('09), Bonnet ('12), and other European anatomists also have supported this view. In this country, Huntington and McClure, with their associates and students, have, until quite recently stood practically alone as sponsors of the local-origin theory.

Differences of opinion among European anatomists regarding these two views have been concerned primarily with the development of blood-vascular channels. The apparent reason for this is that until recently no consistent and uniform plan of development for the lymphatic system had been observed; therefore it was not possible to consider its development in the light of one or the other view. An examination of any one of the current text-books of embryology published prior to 1902 clearly shows how little was really known regarding the development of the lymphatic system up to that time. The general consensus of the opinions expressed, however, seemed to favor the local-origin view.

So far as I am aware, Florence Sabin was the first to emphasize a theory of development for the lymphatic system which could be consistently interpreted in the light of one of these two opposing views. In 1902 Sabin published her first paper on the development of the lymphatic system, in which its development was interpreted in the light of the angioblast theory, or the theory of continuous growth, which is opposed to the local-origin view. The significance of this paper lies in the circumstance that it presents in concrete form a theory of lymphatic development which, if proved to be correct, would seemingly confirm the angioblast theory of His, and would thereby establish the theory of a uniform plan of development common alike to all intraembryonic blood-vessels and lymphatics. The appearance of this paper may be said to have served as a stimulus to other investigators for the publication of a series of investigations extending over a period of fifteen years which, at first, were primarily concerned with the development of the lymphatic system, but which later inevitably included the development of the blood-vascular system as well.

By injecting India ink at definite points into the subcutaneous tissue of a consecutive series of pig embryos, Sabin attempted to show that all lymph vessels bud off from the veins at four primary centers and then invade the skin, as well as the deeper-lying regions, by a process of centrifugal growth. She states (p. 387):

It has now been shown that the lymphatic system in the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction which the endothelial bud takes as it grows from the vein. In the ducts themselves there are no valves at first. From these two buds and later from two similar buds in the inguinal region ducts grow toward the skin and widen out to form four sacs or lymph hearts and from these sacs the lymphatics grow to the skin and cover its surface. At the same time there is a growth of ducts along the dorsal line following the aorta to make a thoracic duct from which the lymphatics grow to the various organs. Thus the ducts of the lymphatic system gradually invade the body, but there are certain tissues which they never reach in the adult, for example, the cornea and cartilage.

It is evident that this theory of a continuous centrifugal growth of lymphatic endothelium from the endothelium of the veins is merely an extension and application to the lymphatic system of the angioblast or ingrowth theory of His. Viewed in the light of the angioblast theory, the endothelium of the lymphatics, like that of the blood-vessels, would possess a marked specificity. It would never arise discontinuously in situ from mesenchyme, but invariably in continuity with some preexisting endothelium whose origin could also be traced back directly and continuously to a unit vascular anlage known as the angioblast.

In 1906 Huntington and McClure read before the Association of American Anatomists a paper in which they claimed that the main lymphatic vessels of the cat do not appear to develop from the endothelium of the veins by a process of centrifugal growth, as maintained by Sabin, but develop in situ from mesenchyme. They also showed that certain of the main lymph channels of the cat, such as the thoracic duct, assume in the adult, a position, occupied in the embryo by functional venous channels which later undergo degeneration. This paper was published in volume 1 of the *Anatomical Record*, which appeared in 1907.

Following Sabin's first paper in 1902, nearly one hundred others have appeared in substantiation of one view or the other. Since 1906, the investigations concerning the histogenesis of endothelium, with the exception of an important paper by Hahn in 1909 and a very few others, have all been made by American anatomists. The following methods have been employed in the investigation of the problem:

1. The injection method;
2. Study of sections and reconstruction of uninjected embryos;
3. Study of sections and reconstruction of injected embryos;
4. Study of the living embryo;
5. Experimental method:
 - a. Isolation of parts of the embryo which are allowed to develop independently;
 - b. Chemical treatment of the embryo;

c. Study of the reaction of endothelium toward colloidal acid dyes in the living embryo (so-called method of vital staining).

While all of the papers published in connection with this problem are important in the sense that they have somewhat influenced its solution, still there are certain ones among them that have had a peculiar importance in directing the course of the investigation. It is to this latter group of papers that we shall for the most part give our attention.

In 1905, slightly previous to the first publication by Huntington and McClure, a paper by F. T. Lewis appeared on the development of the lymphatics in the rabbit. Lewis described the main lymphatics as being developed through the confluence of independent endothelial-lined sacs which he thought had become detached from the veins. While this paper appeared to coincide with the view that the lymphatics were derived from the endothelium of the veins, it also emphasized the circumstance that the main lymph channels did not grow out continuously and centrifugally from the endothelium of the veins, but were formed by the confluence of a number of independent anlagen.

This paper by Lewis influenced greatly the subsequent work of certain investigators. The angioblast theory and Sabin's conception of the development of the lymphatics demanded that these independent lymph sacs of Lewis should still remain in continuity with the veins, regardless of the fact that such continuity could not be actually demonstrated. In a paper published in 1908, Sabin, while finally recognizing the existence of these endothelial-lined lymph sacs of Lewis, asserted that they appeared isolated only in the study of serial sections, and still firmly maintained that their continuity could be demonstrated by the method of injection.

In 1908 Huntington and McClure read a preliminary paper on the development of the jugular lymph sacs in the cat. This paper appeared in its completed form in 1910. They observed that the jugular lymph sacs of the cat did not bud off from the veins in the neck as two blind ducts, as maintained by Sabin, but were derived from a preceding plexus of vessels which, at a number of points, communicated with the veins of the neck.

This plexus of vessels, out of which each jugular lymph sac consolidated, was termed a venolymphatic plexus, on account of its duplex relation to the lymphatics and veins. The investigation led Huntington and McClure to conclude at that time that the jugular lymph sacs were of venous origin and that they formed reservoirs, comparable to the lymph hearts of the lower vertebrates, into which the independently formed systemic lymphatic vessels opened before draining into the veins. The acknowledgment that the jugular lymph sacs were of venous origin seemed to the adherents of the angioblast theory additional evidence that the local origin theory was incorrect.

In 1908 McClure published also a paper on the development of the thoracic duct in the cat, in which he accepted the view advanced by Lewis, that the thoracic duct was developed through the confluence of a multiple series of endothelial-lined vesicles which had become detached from the veins. This view he later retracted in 1910.

In 1909 E. R. Clark published the first of a series of papers on the growth of the lymphatics in the living tadpole's tail. He observed that the lymphatics grew by a process of sprouting or budding from preexisting lymphatic endothelium, and also clearly showed that in such cases contiguous mesenchyme cells were not involved in the process. Although Clark did not describe any instance in which the lymphatics were found to bud or sprout from the endothelium of the veins, he believed that all lymphatics of the body were formed by a process of sprouting, and inferred accordingly that they originally sprouted from the endothelium of the veins. Clark's papers were regarded by the adherents of the angioblast theory as furnishing the final proof in favor of this view.

1909 marks also the beginning of the revival of the study of the development of the intraembryonic blood-vascular system. By the aid of the injection method, Evans concluded that a united vascular system was always present in the embryo, so that blood-vessels form a single though irregularly branched endothelial tree whose branches are in no case added after an independent formation, but arise always by sprouting from the parent trunk.

This view, as expressed by Evans, coincided with the angioblast theory of His.

In 1910, at the International Anatomical Congress in Brussels, papers were read by Huntington and McClure on the development of the thoracic duct and the mesenteric lymphatics in the cat. The former paper was published in 1911 by Huntington in the form of an extensive monograph. In the latter paper McClure formally retracted the view previously expressed in 1908 that the thoracic duct was derived from the endothelium of the veins. These two papers described in greater detail than had hitherto been given the local origin from mesenchyme of the thoracic duct and mesenteric lymphatics.

With the exception of the work of E. R. Clark, the evidence for a venous origin of the lymphatics had up to this time been based almost exclusively upon the results obtained by the use of the injection method. This was the case with the American investigators, as well as with most members of the Polish School, led by Hoyer. Huntington and McClure had frequently called attention to the self-evident fact that the method of injection, even if successful, would only demonstrate the channels or spaces actually continuous with each other at the time the injection was made, and would completely fail to reveal vascular spaces, as yet independent of those injected, even though a connection between them might subsequently be formed.

This inadequacy of the injection method led to a more detailed study of serial sections of injected embryos in order to determine whether independent anlagen of the lymphatic system were present beyond the injected field. Stromsten was the first to analyze the subject critically from this standpoint in a series of three papers, the first one of which appeared in 1910. He injected the lymphatic system in a consecutive series of turtle embryos, and asserted that independent anlagen of the lymphatics were invariably present beyond the injected field.

With the exception of a paper by Huntington on the development of the lymphatics in reptiles, no actual advance in any direction, not already mentioned, was made in 1911.

Evidence in favor of the venous origin of the lymphatics and of the angioblast theory in general had apparently reached the high-water mark in 1911. Articles by Evans and Minot on the blood-vascular system, and by Sabin, on the lymphatics, appeared in Keibel and Mall's *Embryology*, definitely declaring in favor of the specificity of endothelium and the angioblast view. Also, two papers of a critical nature regarding the methods thus far employed by different investigators were published by Sabin and E. R. Clark in 1911.

In 1912 an investigation appeared by Kampmeier, who demonstrated the presence of independent anlagen of the thoracic duct in an injected pig embryo, which had been loaned to him by Sabin with the express purpose of furnishing evidence that such anlagen did not exist. A critique of this paper was published by McClure in 1912. Also in 1912 Sabin arrived at the conclusion that the thoracic duct was formed in part by a down growth from the jugular lymph sac, and in part through a fusion of independent endothelial-lined sacs, which, like Lewis, she still regarded, however, as detached veins. With the exception of a general review of the subject which appeared in 1913 and the Harvey lecture on the growth of the lymphatic system published in 1915, this is the last investigation, so far as I am aware, which has been published by Sabin on the development of the lymphatic system.

In 1912, E. R. and E. L. Clark published a series of observations on the living growing lymphatics in the embryo of the domestic fowl, and concluded that the posterior lymph hearts were derived from the veins. A paper on the development of the jugular lymph sac in the domestic fowl appeared also in 1912 by A. M. Miller, in which he claimed that the jugular lymph sac developed in situ from mesenchyme.

In 1912, Bremer also attempted to strengthen the angioblast theory by showing that the discontinuity described for the developing intraembryonic blood-vascular system did not really exist, as these apparently discontinuous elements were connected with one another by solid angioblast cellular cords.

Another paper of importance appeared in 1912 by Whipple and McWhorter, who were the first to follow in detail the development of the blood-vessels on the yolk-sac in the living embryo of the chick. They were able to demonstrate and to record photographically that the blood-vessels in the area pellucida were formed through the concrecence of separate and locally formed anlagen, and that the growth of endothelium was not continuous between the yolk-sac and the body-axis of the chick.

Between 1913 and 1915, McClure published a series of papers on the development of the lymphatics in fishes. In a consecutive series of injected trout embryos, he found that the main lymphatic channels were formed through a confluence of separate and independent endothelial-lined vascular spaces, or lymph vesicles, which could not be injected from the veins. Two of these independent anlagen, the subocular lymph sacs, were relatively huge structures which could be observed in the living embryo. By injecting India ink into these sacs in the living embryo it was observed in early stages that ink did not pass out of the sacs into the other portions of the lymph system or into the veins. A study of the development of these subocular lymph sacs showed that they develop in situ from mesenchyme; also that they only temporarily retain their independence as disconnected anlagen of the lymphatic system, and secondarily establish a connection with other independent lymph vesicles to form a continuous system of vessels, through which the lymph can then drain into the veins.

The chief significance of this investigation lies in the circumstance that one is actually able to demonstrate in the living trout embryo the presence of independent and discontinuous anlagen of the lymphatic system.

In 1913, Miller published a paper on the development of the thoracic duct in the domestic fowl. He observed that the periaortic mesenchyme of the chick is the site of a most active and abundant intraembryonic haemopoiesis in situ. He showed that masses of developing blood-cells differentiate axial strands around the aorta directly from the indifferent periaortic mesenchyme. Subsequently, the anlagen of the thoracic ducts appear

in this periaortic area as isolated intracellular clefts and spaces. These spaces become confluent, receive the blood-cells developed in the mesenchymal blood-islands, and convey them through the channels of the thoracic duct to the jugular lymph sacs, and through them into the circulating venous blood-stream. After this evacuation of their early blood contents, the axial lymphatic channels are retained as the permanent thoracic ducts. Lymph vessels which convey developing blood-cells to the circulating blood-stream, Miller designated as haemophoric lymph vessels.

The presence of haemophoric lymph vessels in the embryo of the domestic fowl led Huntington to make a further detailed study of the development of the jugular lymph sacs in the cat, in the early embryonic stages of which blood-cells had previously been observed by McClure and Huntington in certain disconnected anlagen.

In a paper published in 1914 Huntington showed, by means of an improved technique, in stages earlier than those hitherto studied by him and McClure, that the anlagen of the jugular lymph sacs, like those of the thoracic ducts in the chick, first appear in the midst of an extensive haemopoietic field as isolated intercellular clefts or spaces. When these spaces become confluent to form the jugular sacs, the walls of the latter enclose great masses of blood-cells. The jugular sacs then temporarily connect with the veins at a number of points at which the blood-cells are evacuated into the blood-stream. After the evacuation of this blood content, each jugular sac temporarily becomes detached from the veins, but later establishes a connection with them at two typical points.

Huntington also showed that in the embryo of the cat the primitive ulnar lymphatic functions temporarily as a haemophoric vessel, which later undergoes complete atrophy. This investigation by Huntington emphasized two important points: first, that the presence of blood-cells in the lymphatics does not necessarily signify a venous origin of the latter, and, second, that the jugular lymph sacs of the cat, like the main systemic lymphatics, are developed in situ from mesenchyme.

Huntington's paper was followed by a preliminary account by West on the development of the posterior lymph heart in the domestic fowl. The final paper was published in 1915. West asserted that the posterior lymph heart of the domestic fowl develops in situ from mesenchyme and, like the thoracic ducts of the embryo, possesses a haemophoric function during the early stages of development. The results of this investigation were not in accord with those previously obtained in 1912 by the Clarks who maintained that the lymph heart was derived from the veins.

Two papers appeared in 1914 on the development of the intraembryonic haemal endothelium by Schulte and Bremer, respectively. Schulte's publication appeared to be even more convincing than any morphological investigation hitherto published which had dealt with the genesis of intraembryonic haemal endothelium. He proved in a most decisive manner that the umbilical vein of the domestic cat develops in situ from mesenchyme, and is not derived from yolk-sac angioblast. In his 1914 paper Bremer modified his earlier conception of the angioblast theory. He claimed that the angioblast might also arise in the embryo independently of that on the yolk-sac, its only prerequisite being that it shall grow continuously from one or many sources of origin through the embryonic tissue as solid cords of cells. This later view of Bremer's could be interpreted as a recognition of the principle involved in the theory of the local origin of endothelium, as this theory does not deny the possibility of the growth of endothelium after it has once been locally formed.

1914 marks the termination of a period in which the investigations were almost exclusively morphological in character, and the beginning of one in which certain of these investigations have been confirmed from the standpoint of experiment.

It will be observed that from 1914 up to the present writing but two investigations have appeared in print in which a claim has been made that intraembryonic haemal endothelium is derived from yolk-sac angioblast (Wislocki, '16) or that lymphatic endothelium is a derivative of the endothelium of the veins (Wislocki, '16; E. R. and E. L. Clark, '20).

From 1914 on, the question of the specificity of endothelium in particular, and that of the continuous growth theory of endothelium in general, has seemed to lack its former support.

Two important experimental papers appeared in 1914 by W. C. Clarke and by Miller and McWhorter, respectively. Clarke's paper was concerned especially with the question of the specificity of endothelium. He showed that vascular endothelium, wherever encountered, haemal or lymphatic, may be an instance of the environmental adaptation of an originally isodiametric mesenchymal cell subjected to mechanical influence. He observed that unilateral pressure, e.g., the accumulation of fluid under tension in intercellular spaces, will produce flattened modified mesenchymal cells which resemble endothelium. If the pressure is released, the former endothelial cell will promptly revert to the type of the indifferent mesenchymal cell, of which it is merely an adaptive form, modified in accordance with definite hydrostatic and other purely mechanical factors. Consequently, he claimed that the modified mesenchymal endothelial cell loses all pretensions to specificity in the sense implied by the angioblast theory.

Miller and McWhorter cut off the lateral half of the entire area opaca of the blastoderm in developing chick embryos at a time when no blood-vessels or so-called angioblast had appeared in the area pullucida or embryonic body, and the blastoderm was then allowed to proceed in development. After a period of incubation lasting from twenty-four to forty-eight hours, the embryos were sectioned and graphic reconstructions made of the vascular system in the embryonic body. Blood-vessels were invariably found on the operated or injured side as well as upon the uninjured side of the body. Essentially similar experiments had previously been made by Hahn in 1909 with the same results. Hahn's paper had not been brought to the attention of Miller and McWhorter, however, until after their own experiments had been completed. The results of both sets of experiments seemed to leave little doubt as to the validity of the view that the intraembryonic blood-vessels are not derived from yolk-sac angioblast, but arise *in situ* within the body-axis of the embryo.

Following the publication of Miller and McWhorter's paper, the objections urged against it from certain quarters included the following: the incisions may not have been made sufficiently close to the embryonic axis or may not have been made sufficiently early and endothelium may have grown in from the opposite side or from the ends. In order to satisfy these objections, Reagan was able in 1915 to isolate completely a portion of the chick's embryonic body from all the outlying blastoderm at a time before the embryonic tissue is vascularized. After a period of incubation he found that endothelial-walled vessels or spaces were invariably present in such meroplasts.

In 1915, Stockard observed in chemically treated living teleost embryos, in which development of the vascular system had been arrested, that vascular endothelium arises in loco in many parts of the embryonic body. Stockard also observed in the living embryo of *Fundulus* the active migration of mesenchyme cells from the body-axis on to the yolk-sac, where they entered directly into the formation of the endothelium of blood-vascular channels.

Similar results were subsequently obtained also by Reagan in 1915-17 in hybrid teleost embryos and in normal and chemically treated living embryos of *Fundulus*.

In 1915 McClure published a critical review of the investigations on the histogenesis of the vascular system which had appeared up to that time.

In 1916, following the method employed by Stockard and Reagan, McClure observed in chemically treated living embryos of a teleost (*Erymizon*) that development of the lymphatic and blood-vascular channels could be arrested at a stage in which both were represented by completely independent and discontinuous anlagen. This investigation by McClure on teleost embryos showed that the local origin of lymphatic endothelium from mesenchyme, like that of the blood-vessels, could be determined by experiment. While this investigation has been completed, it has not as yet been published in full.

On the basis of the discovery that trypan blue is an elective stain for amphibian embryonic lymphatic endothelium, Wislocki

claimed, in 1916, that the different theories of lymphatic development could be subjected to a crucial test. Wislocki held that lymphatic endothelium was derived from the veins.

The 'crucial' tests regarding the theories of lymphatic development referred to above by Wislocki were made by McClure and published in 1918 in *The American Anatomical Memoirs of The Wistar Institute of Anatomy*. As stated by McClure: Evidence from the standpoint of vital staining by colloidal acid dyes that the lymphatics grow out from the veins, would at least call for a demonstration of an early stage of growth, at which a vitally stained lymphatic is in the process of growing out from a vein. I may state that not even the faintest approach to such a demonstration can possibly be realized in the embryo of either the frog or the toad. The reason for this is plain. The lymphatic system of the frog and the toad does not become vitally stained, so to speak, by colloidal acid dyes, until an extensive system of continuous lymphatic vessels have been formed, which convey lymph from practically all regions of the body to the anterior lymph hearts and thence to the veins. It is also significant to note that the endothelium of the anterior lymph hearts in the frog and toad, from which, by some investigators, the lymphatics are supposed to grow, is not vitally stained by colloidal acid dyes. As a matter of fact, the earliest stage of development at which the lymphatics react toward these dyes in the frog and toad is one not very far removed from their permanent larval form. Such an advanced stage would not ordinarily be regarded as a favorable one from which to infer in what manner the lymphatics have been formed.

The method of vital staining by colloidal acid dyes, like the injection method, as ordinarily used, does not therefore demonstrate the existence of lymphatics in the embryonic body until their development is far advanced and until continuous channels are formed. This method, like the injection method, is incapable of demonstrating the early independent anlagen of the lymphatics, like those of the head sinuses in the frog and toad, which at one time are entirely independent of each other and of the veins. The 'crucial' test referred to by Wislocki therefore completely

fails, as a vital staining of the lymphatic tissues by his method is not possible at a time when the earliest anlagen of the lymphatic system first arise.

It was also shown that lymphatic endothelium is not the only tissue which reacts toward colloidal acid dyes. The reaction is therefore not specific in the sense implied, but rather an attempt on the part of the lymphatics and certain other tissues to rid the blood-stream of the dye.

In 1917, Sabin definitely declared in favor of the local origin of intraembryonic haemal endothelium—a view which she opposed in 1913. Her present position regarding the development of the lymphatics, so far as one is able to judge, is similar to that stated by her in 1913. In 1920 she published also an elaborate monograph which appeared in the Mall Memorial Volume, in which she described in considerable detail the local origin of intraembryonic blood-vessels and red corpuscles as observed in the living embryo of the chick.

If we analyze the results of the above-mentioned investigations, it is evident that the morphological evidence favoring the general principle of a local origin of intraembryonic endothelium from mesenchyme has been completely confirmed by experiment, and that the angioblast theory, in the sense maintained by His, therefore no longer holds.

While differences of opinion may still exist, as regards details of the process, both for the lymphatic and blood-vascular systems, it is plain from this brief sketch, that the general principle of the local genesis of intraembryonic endothelium from mesenchyme, a theory so recently and so vigorously opposed by a large group of American anatomists, may now be regarded as an established fact.

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Resumen por el autor, C. V. Cowdry.

La tendencia conservadora en la nomenclatura citológica.

El autor considera con cierta aprehensión la multiplicidad creciente de los términos citológicos. Un análisis demuestra que algunos de ellos han sido introducidos para indicar ciertos atributos morfológicos; otros para designar ideas relativas a la constitución química; otros, de nuevo, para recordar los descubridores (introduciendo de este modo todo el problema de la prioridad) mientras que los más confusos han sido ideados para proclamar alguna interpretación funcional más o menos teórica. La confusión resultante es perjudicial para el pensar claro y retarda definitivamente el progreso de la citología, pero ha de empeorar aún más hasta que los investigadores se hagan cargo suficientemente de la situación, considerando la introducción de nuevos términos sin buenas razones, como una especie de ofensa. Un cierto grado de tendencia conservadora es necesario. El autor indica que sería útil que alguna asociación nacional determinase los principios que han de inspirar la nomenclatura citológica para uso de los investigadores.

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CONSERVATISM IN CYTOLOGICAL NOMENCLATURE

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Repeated and ill-advised invention of new terms to designate cytoplasmic structures has resulted in considerable confusion.

A pathologist, for instance, who wants to review the literature on mitochondria is apt to be discouraged when told that, in order to be on the safe side, he must look for papers under at least a dozen different headings: bioblasts, chondriosomes, plastochondria, etc.

It is not without reason that biochemists and physiologists, who are able to help so much, are content to remain ignorant of advances in cytology. They are confronted by a mass of almost meaningless words which cannot be defined with anything approaching the accuracy to which they are accustomed. All too frequently they meet hypotheses which are absurd in the light of recent advances in physics and chemistry. A barrier is built up where the closest coöperation is needed. New and delicate methods are being devised for the measurement of temperature, electrical potential, ionization and other phenomena, which are of the utmost value to cytologists, but which their limited knowledge of physics and chemistry prevents them from using. Not infrequently the inventors themselves are unaware of the nature of the problems for which they are unconsciously supplying the means of solution.

The cytoplasmic material commonly called mitochondria (Benda, '98, p. 397) presents certain general properties, though the fact that it varies slightly in different cells suggests that it is in reality a mixture of chemical substances:

1. It may be easily seen to occur in living cells in the form of granules, rods, and filaments, and occasionally of networks, which vary in shape and in number in different conditions.

2. It gives a characteristic color reaction in living cells when janus green B (diethylsafraninazodimethylanilin) is applied in a solution as weak as 1:500,000. It first assumes a bluish-green color, then turns pink (diethylsafranin), and finally bleaches to the leucobase. The delicacy of the reaction is shown by the fact that janus green (Grübler) and janus green C will not stain mitochondria specifically, though they differ only in the substitution of an H₂ or (CH₃)₂ group in place of the (C₂H₅).

3. Its solubilities are characteristic, and in fixed tissues it stains more or less specifically by the methods of Altmann, Benda, Bensley, and others.

The term 'mitochondria' is well known and has been widely used for over twenty years. It indicates variability in morphology, from threads to granules, and carries no theoretical or functional interpretation whatsoever (Cowdry, '18, p. 47).

Mitochondria did not altogether escape the older authors, who, with their relatively crude methods, observed and described them, often in confusion with materials of quite different nature, under many terms of which the following may be mentioned:

Archoplasma-schleifen (Hermann)	Neurosomen (Held)
Basal filaments (Solger)	Nucleus of Balbiani (Van der Stricht)
Centrophormien (Ballowitz)	Plastidulen (Maggi)
Chromidia ¹ (Hertwig)	Plasmosomen and Plasmomiten (Arnold)
Cytomicrosomes (Heidenhain)	Protoplasma supérieur (Prenant)
Ergastoplasme (Garnier)	Pseudochromosomen (Heidenhain and Van der Stricht)
Fila (Flemming)	Q and J granules (Holmgren)
Fuchsinophile granules (Galeotti and others)	Sarcosomen Retzius
Granula (Metzner)	Sphären (Rhode)
Granules (of Schridde)	Sarcosomen (Retzius)
Interstitial-Körner (Henle and Koelliker)	Sphéroplastes of Protozoa (Fauré-Fremiet)
Leucoplastids (Schimper)	Vermicules (Laguesse)
Microsomes (Van Beneden)	Vegetative filaments (Altmann)
Mitom (Flemming)	Vitellogene Schicht (Van der Stricht)
Nebenkern (Butschli)	
Nematoplasten (Zimmermann)	

¹ In the days of confusion with 'chromidia' it is highly probable that mitochondria were described under this heading, though, in reality, they are wholly different.

Since their recognition as a definite class of cell granulation the literature has been flooded with new terms introduced by the authors with the best intentions and, in each case, in the firm belief that they would finally clarify the situation:²

Benda ('98, p. 397)	Chondriomitom	A feltwork of mitochondria
Benda ('99, p. 382)	Chondriomiten	Thread-like mitochondria
Benda (Van der Stricht, '04, p. 105)	Chondriorhäden	Shaft-like mitochondria
Benda (Van der Stricht, '04, p. 105)	Chondriosomen	A generic term to include all forms of mitochondria
Benda (Van der Stricht, '04, p. 105)	Chondriosphären	Spherical mitochondria
Renaut and Dubreuil ('06, p. 230)	Perinème	Mitochondria as they occur in certain connective tissue cells
Renaut and Dubreuil ('06, p. 230)	Pericaryonème	Mitochondria as they occur in certain connective tissue cells
Koltzoff ('06, p. 468)	Mitogels	Large mitochondrial masses formed by confluence
Koltzoff ('06, p. 468)	Mitosols	Mitochondrial droplets
Koltzoff ('06, p. 468)	Mitochondrosols	Mitochondrial droplets
Duesberg ('07, p. 284)	Mitochondrial apparatus	A general term, same as chondriom (Duesberg, '08, p. 261)
Meves ('07, p. 401)	Chondriokonten	Rod-like mitochondria
Meves ('07, p. 403)	Chondriom	Cytoplasmic content of mitochondria
Giglio-Tos and Granata ('08, p. 14)	Chondriotaxie	The arrangement of granular mitochondria in threads
Giglio-Tos and Granata ('08, p. 1)	Chondrioderese ³	Division of mitochondria
Regaud ('09, p. 921)	Eiectosomes	To indicate supposed property of picking up substances from the surrounding medium and elaborating them
Meves ('10, p. 150)	Plastosomen	Generic term to include all forms of mitochondria
Meves ('10, p. 150)	Plastochondrien	Granular mitochondria supposed to play a definite part in histogenesis

² Rosenstadt's ('18, p. 193) 'tetrasomen' terminology is difficult to correlate.

³ Comes (p. 423) claims priority over Giglio-Tos in the invention of this term. Since, however, he confuses the mitochondria with the reticular material, it is not clear under which heading it should be given.

Meves ('10, p. 150)	Plastochondriomiten	Rows of granular mitochondria
Meves ('10, p. 150)	Plastoconten	Long rod-like mitochondria
Laguesse ('11, p. 276)	Ergastidions	To indicate supposed relation to ergastoplasm
Romeis ('12, p. 139)	Chondriolysis	Solution of mitochondria
Comes ('13, p. 423)	Condrio-cinesi	Changes in mitochondria during cell division
Comes ('13, p. 423)	Condrio-dieresi	Changes in mitochondria during cell division
Comes ('13, p. 423)	Idio-condrioma	Mitochondria supposed to play a definite part as carriers of heredity
Comes ('13, p. 423)	Trofo-condrioma	Mitochondria supposed to be concerned in nutrition
Asvadourova ('13, p. 293)	Chromochondries	To indicate supposed part in pigment formation
Wildman ('13, p. 428)	Karyochondria	To indicate supposed nuclear origin
Ciaccio ('13, p. 725)	Plastolysis	Solution of mitochondria
Ciaccio ('13, p. 725)	Plastorhexis	Breaking up of mitochondria into granules and vesicles
Ciaccio ('13, p. 725)	Präplastorhexis	Changes preliminary to plastorhexis
Champy ('13, p. 157)	Chondrioplastes	To indicate supposed part in formation of plastes
Arndt ('14, p. 55)	Caryosomochondria	To indicate supposed origin from the caryosome
Jordan and Ferguson ('16, p. 94)	Myochondria	To indicate supposed mitochondrial origin of myofibrils
Gatenby ('18, p. 223)	Micromitochondria Macromitochondria	Small mitochondria which are less dense than the larger ones and behave differently in the spermatogenesis of certain invertebrates
Gatenby ('18, p. 210)	Macromitosome	Used synonymously with the mitochondrial coil (nebenkern or paranucleus) of Lepidoptera
Meves ('18, p. 273)	Chondriomer	Suggests termination 'er' for purely morphological nomenclature
Guilliermond ('19, p. 239)	Mitochondries elaboratrices	To indicate mitochondria supposed to differentiate into chondrioplastes in the animal cell and into plastides in the vegetable cell

Guilliermond ('19, p. 239)	Mitochondries vegetatives	To indicate non-functional mitochondria which are thought to perpetuate the chondriome
Dangeard ('19)	Plastidome	(Quoting from Guilliermond '21, p. 73)

The reticular material cannot be so precisely defined, but we do know that under certain conditions it (or its mordant) has an affinity for silver; that after long immersion it blackens with osmic acid; and that, when fixed by appropriate methods (Bensley, '10, p. 191), it presents the form of a system of clear uncolored spaces. It may also be recognized by its morphology and by its position in the cell. Golgi ('98, p. 1) was the first to refer to its reticular shape. By substituting the general term 'material' in place of 'apparatus,' the idea of a definite system of canals is not sustained (Cowdry, '21, p. 8).

Like the mitochondria, it was occasionally seen by the older authors and described, together with other materials under many headings of which I have selected the following:

Archoplasm (Boveri)	Nebenkern (Butschli)
Boyaux incolorés (Van Beneden)	Primitivröhrechen (Nansen)
Centrodeutoplasm (Erlanger)	Vasa serosa intracellularia (Adamkiewicz)
Cytosoon (Gaule)	Zentralkapsel (Heidenhain)
Mitosome (Platner)	

Stimulated by the discoveries of Golgi, Holmgren, and Kopsch, new terms are being devised wholesale:

Holmgren ('99, p. 139)	Saftkanälchen	Reticular material as clear canals (negative picture)
Nelis ('99, p. 102)	Spiremes	Reticular material in the form of clear, uncolored spaces
	Etât spiremateux du protoplasma	Reticular material in the form of clear, uncolored spaces
	Bandes incolorés	Reticular material in the form of clear, uncolored spaces
Kopsch ('02, p. 934)	Binnennetz (Netzapparat)	Blackened reticular material
Holmgren ('03, p. 9)	Trophospongium	To indicate canals which penetrate the cell from the exterior. Evidently not the reticular material, which is purely cytoplasmic, though sometimes applied to it.

Cajal ('04, p. 25)	Aparato tubiliforme	Same as 'red endocellular de Golgi'
Fuchs ('04, p. 501)	Fadenknauel	Same as Saftkanälchen or reticular material in the form of clear, uncolored spaces
Sánchez ('07, p. 167)	Appareil réticulaire de Cajal-Fusan	Homologue of 'Appareil de Golgi-Holmgren'
Cajal ('08, p. 123)	Conduits de Golgi-Holmgren.	Reticular material as clear canals
Perroncito ('09) (Duesberg, '11, p. 884)	Dittocinesi	To indicate the process of breaking up of reticular material into fragments during mitosis
Bensley ('10, p. 179)	Dittosomi Canalicular apparatus	The individual fragments Reticular material as clear canals
Maccabruni ('10, p. 447)	Retzius-Holmgren-schen Kanälchen	To indicate tubular nature and discovery by Retzius and Holmgren
Kuschakewitsch ('13, p. 310)	Sphärosomen Sphärotheca	Reticular material during a phase of spermatogenesis
Terni ('14, p. 68)	Formazioni periid- iozomici	Reticular material grouped about the centrosome
King (Gatenby, '16, p. 418)	Acroblasts	"Dietosomes or individual units of the Golgi apparatus" (Gatenby and Woodger, '21, p. 279)
Stockard and Papanicolaou ('18, p. 39)	Idioectosome Idiophthartosome	Reticular material as it appears in certain stages of spermatogenesis
Gatenby ('18 a, p. 403)	Chondrioplasts	Same as 'Golgi rods' (i.e., rod-like masses of reticular material)
Penfield ('20, p. 303)	Retispersion	To indicate dispersion of the reticular material to the cell periphery
Penfield ('20, p. 303)	Retisolution	Solution of the reticular material
Saguchi ('20, p. 388)	Intracellular network	"The apparatus which can be made manifest by various methods, such as Kopsch's and Wiegler's osmium method, Sjøvall's formalin-water-osmium method, Cajal's uranic nitrate-silver method, and Golgi's arsenic method"
Saguchi ('20 a, p. 15)	Urano-argento- phile apparatus	"Filamentous or granular corpuscles which can be made manifest by the Cajal uranic nitrate method"

The so-called 'chromidal substance' is another cytoplasmic constituent of wide distribution encountered by many investigators, who, failing to realize the substantial similarity of the fundamental vital processes in different tissues, have called it all kinds of names. Specific granulations, occurring only in certain cell types, give us much less trouble.

We cannot take refuge in priority, which is very difficult to establish, because modern cytology is resolving itself more and more into a gradual approximation to a logical interpretation of living materials observed for the first time years ago with the aid of Abbe's condenser and the newly introduced apochromatic lenses. Newly recognized protoplasmic constituents are, for the most part, specific in nature, like secretion antecedents, not general in distribution, like the mitochondria, and are only revealed by refinements in microchemical technique. It is but natural that investigators should introduce terms indicative of their theoretical conceptions, but the habit is a bad one, because our ideas are changing so rapidly. Altmann's choice of the term 'bioblasts,' to indicate his belief that small fragments of material, which we now know to be mitochondria, were independent micro-organisms, attracted so much criticism and ridicule that his valuable discoveries were soon forgotten and investigations along this line were set back many years. Terms of this kind indicative of the supposed functional activities of cellular structures are often invented one year only to be cast aside the next, but they remain in the literature to mystify visitors from other sciences.

I question also the wisdom of using special terms to indicate slight differences in morphology, which after all can only be detected with oil-immersion lenses. In the case of mitochondria, we are dealing with fragments of material of microscopic size. Changes in form occur from moment to moment and are merely the expression of interaction between the mitochondria and the surrounding medium; only rarely do they indicate noticeable differences in chemical constitution.

Realizing that the cell is a living unit, which is changing continually, our terms should, it appears to me, be general and comprehensive, not limited and specific. While the materials in

question continue to defy chemical analysis, the probability that they undergo progressive and important chemical changes, which are not revealed by our imperfect methods, should be entertained, and speculations as to their physiological rôle should be advanced with caution. Most of the structural differentiations recognized by cytologists are mixtures, not pure chemical substances, so that the problem is by no means a simple one.

Perhaps the greatest difficulties are met with during cytomorphosis. In spermatogenesis, for instance, the mitochondria appear to change into some kind of material fairly resistant to acetic acid and of different morphology and staining reaction. At what stage should we cease calling them mitochondria and speak only of the product, whatever it may be? The same difficulty is met with in the case of the reticular material. The introduction by Stockard and Papanicolaou ('18, p. 39) of the following terms, in connection with the development of the idiosome, is, in my opinion, of questionable value: *calyptosome*, *karyogranulomes*, *idiogranulotheca*, *idiophthartosome*, *idiosphaerosome*, *idiosphaerotheca*, *cytogranulomes*, *cryptosome*, *idioectosome*, *idiocendosome*, *idiogranulomes*, *spermiocalyptrotheca*. I am inclined to agree with Duesberg's ('20, p. 8) usage in this connection. Since we are ignorant of the chemistry of the cytomorphic change, we have to come to some arbitrary decision in each instance, for every cell in the body as it grows old presents the same problem with respect to all its structural components.

So many and so varied are the structures which can be recognized in the nucleus and cytoplasm in normal as well as pathological conditions, the chemistry and physics of which we know next to nothing, that hundreds of terms are in every-day use, many of them synonymous. Specialization is carried so far that students of the cytoplasm become bewildered when first they interest themselves in nuclear structure, and investigators familiar with the nucleus have to go slowly in the terminology of leucocytic granulations and secretion antecedents, if, indeed, they ever consider them.

Our main problem is not an etymological one, but confusion in nomenclature reflects inaccuracy in methods of cytologic

analysis and in the coördination of our knowledge of cellular physiology. It arises from the fact that our information regarding the chemistry of protoplasm is largely indirect and deducive. With the discovery of new methods of direct analysis and the strengthening of the mechanistic point of view, that we must try to explain vital phenomena in terms of physics and chemistry, investigators will be less ready to advance theories without carefully considering the chemical changes which they hypothecate, or to make unqualified statements of the transformation of one cytoplasmic material into another without knowing the constitution of either and the probability or improbability of such a change. With more accurate knowledge will come conservatism; the useful terms will gradually be sorted out and the bad ones consigned to oblivion. But this rapprochement between cytology and the more exact sciences will be a slow process extending over many years. In the meantime are we to follow the command of the old Chinese philosopher, Lao Tzu: "Practise inaction; content yourself with doing nothing"?

Some will say that it is not a matter which warrants group action, that the individual must continue to be a law unto himself, and that the very haziness of our knowledge of cellular structure will make it impossible to arrive at any *modus operandi*. The Basel anatomical nomenclature of visible structural components, a much easier task, has on the whole been of the greatest help. In chemistry, certain conventions in terminology are recognized even in respect to compounds about which very little is known and concerning which there is much speculation.

Individual preference will of course continue to be followed, but I cannot help thinking that an attempt by some national organization, or group of individuals, not to dogmatize further on terms, but rather to determine guiding principles in cytologic nomenclature for the use of investigators would be desirable. To yield the best results this committee or group should consist of students interested in the structure of both the nucleus and the cytoplasm as well as of a botanist, a protozoologist, and a biochemist, because cytology, dealing as it does with the minute structure of living material, is the broadest of biological sciences.

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Resumen por el autor, Charles C. Macklin.

Nota preliminar sobre el cráneo de un feto humano de 43 mm.
de longitud máxima.

El presente trabajo comprende una breve descripción del condrocráneo, esqueleto del arco branquial, vértebras cervicales y huesos de membrana del feto humano núm. 886 de la colección de la Carnegie Institution de Washington, basándose en veintiocho modelos y una reconstrucción de perfil. Las porciones cordal y precordial del tallo central forman un ángulo de 115° . Los centros de preosificación se mencionan. El cartílago supracoccipital esta osificándose excepto en los bordes, de los cuales parten los procesos ascendente y descendente. El autor dá una explicación de la equivocación de Bolk al interpretar esta región. El surco occipitoparietal separa parcialmente la placa parietal de la escama occipital situada debajo. Los arcos neurales de las vértebras occipitales están bastante acusados, existiendo tubérculos yugulares marcados y procesos paracondiloides. El cartílago supracoclear falta. El proceso estiloides está unido con la cápsula por medio de cartílago. El proceso mastoideo es un pequeño nódulo libre. El orificio perilinfático está dividiéndose por los procesos intraperilinfáticos anterior y posterior, que se aproximan. Las alas hipoquiasmáticas son pequeñas. Las comisuras prequiasmáticas son precartilaginosas y los orificios del mismo nombre son relativamente grandes. La comisura alicoclear o línula en vías de desarrollo es completa, pero muy delgada. En la región etmoidal no se encontraron cartílagos paraseptales superiores. El proceso cupular es largo y precartilaginoso. En el meato medio se encuentra un proceso precartilaginoso delgado y largo. El autor ha modelado y descrito el sistema del conducto nasolacrimal del mismo modo que muchas de, las demas estructuras con él relacionadas, tales como el notocordio, nervios principales y ganglios, etc. Se han representado todos los huesos de membrana con excepción del nasal.

PRELIMINARY NOTE ON THE SKULL OF A HUMAN FETUS OF 43 MM. GREATEST LENGTH

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Studies on the developing skull of man and the lower forms have recently gained an impetus through the appearance of several papers. Among the contributions from American laboratories may be mentioned those of Kernan ('16) and Lewis ('20) on the human skull, Terry ('17) on the cat, and Rice ('20) on the lizard. Although a great amount of work has already been done, yet the science of craniogenesis is only in its infancy. Of particular service has been the wax-plate method of reconstruction, and in recent years the refinement of this method represented in the plaster of Paris technique (Lewis, '15) has become a very valuable aid. It is of interest to pursue the study of the development of the human skull not only for its own sake, but even more on account of its relation to the larger field of the development of the head, and to the still larger domain of evolution, and I offer this preliminary note upon the developing skull of a human fetus already well advanced in the scale of differentiation in the hope that it may prove helpful in advancing our knowledge of cranial morphogenesis.

The Embryological Collection of the Carnegie Institution of Washington presents splendid opportunities for the investigation of almost any problem in human antenatal development, and I was fortunate in being able to avail myself of its privileges. I chose for study human fetus no. 886, of 43 mm. greatest length, because it represents a stage sufficiently near that of the 40-mm. fetus which I described some years ago (Macklin, '14) to provide interesting material for comparison, and yet sufficiently far from my earlier specimen to avoid duplication of the work of research. No. 886 was obtained in perfect condition and was exceptionally

well prepared. The sections were cut in the frontal plane at a thickness of 100 μ and the series is practically perfect. The models were done in plaster of Paris, and number twenty-eight. They are faithful reproductions of the original structures.

A model of the entire skull, cervical vertebrae, and cartilaginous branchial arch skeleton was first made at a magnification of ten diameters, and this was used for the grosser studies. The central stem and the right half of the occipital cartilage were also made at the same enlargement. For the examination of the minuter details, special models were made of selected parts, taken from the right side of the skull, and enlarged twenty diameters. In addition to the cartilage, the membrane bones of the right side were modeled, together with the closely related soft parts, as the nerves, vessels, mucous membranes, etc., to be referred to in the description. Profile reconstructions were also made, showing the texture of the frontal and parietal bones, and the relation of the skull to the brain and to the external form.

DESCRIPTION

The skull, in general form, is much like that of the 40-mm. human embryo from the collection of Professor McMurrich, of the University of Toronto, known as 'I^a Toronto,' which I formerly described (Macklin, '14), and which will be referred to in this paper as Ia. No. 886, however, is noticeably less developed than Ia, although the latter is shorter, and this apparent discrepancy is to be explained by the fact that the dimension of Ia was obtained by crown-rump measurement, while in the case of no. 886 it was greatest length.

Development is proceeding most rapidly in the anterior parts, notably in the ethmoidal region, judging from the character of the cartilage and from a comparison of earlier and later stages, as that of Lewis ('20), embryo no. 460, of 21 mm., and Ia.

Indications of future ossification centers

The cartilage is mostly mature, and it is practically a continuous mass. In ten regions (four paired and two unpaired) it is undergoing the change preliminary to ossification, although in no

case is there actual bone formation present. This change in the cartilage is most marked in the case of the center for the supraoccipital cartilage, which is single and involves the entire thickness of the plate, but not the entire width, there being a narrow uncalcified edge along the upper border and a short blunt point (the descending process) projecting downward into the foramen magnum. The paired centers for the exoccipitals are situated in and behind the jugular tubercles. These tubercles are distinct ridges which lie just lateral to the canals for the hypoglossal nerves and run back to the posterior condyloid notches on the border of the foramen magnum. At these notches the cartilaginous change involves the entire thickness of the plate.

In the basioccipital cartilage is a small area of modified cartilage which represents the single center of the basioccipital bone. In the temporal wings the cartilage is still less modified, but paired centers can be made out. The sphenotic centers are just beginning, and the cartilage here, at the outer ends of the alar processes, shows only a very small degree of change. There are paired centers near the lower ends of Meckel's cartilages, and here the perichondrium is ossified and represents part of the mandible.

The *central stem* is bent at the body of the sphenoid, the angle between the chordal and prechordal parts being 115° . It is thus narrower than in no. 460, where my measurement from Lewis's figures shows it to be 125° . The bodies of the cervical vertebrae make with the chordal part of the central stem an angle of 125° . This is probably a more variable angle. The corresponding angle in 460 was 110° .

The *foramen occipitale magnum* is relatively larger than that of Ia, and the tips of the occipital vertebral arches are farther apart, making the superior incisure much wider. These arch tips are the same distance apart as those of the atlas, but as we proceed down the spinal column the arch tips come closer and closer together. Those of the seventh cervical vertebra, however, are still some distance apart. Closure of the cervical spinal canal is evidently following the familiar course, in being completed earlier below than above.

It seems possible that the occipital vertebra may always present a *spina bifida*, the arch tips not coming into actual contact and fusing to complete the foramen magnum behind, as I formerly held. This is undoubtedly the case in certain dogs, notably of the short-nosed type. Dr. Adolph H. Schultz has shown me mature skulls of bulldogs and pugs, nos. 71, 381 and 382 of his collection, where the extremities of the occipital neural arches are separated by a distinct interval and where, accordingly, the superior occipital incisure has persisted. It is a conspicuous notch which projects dorsally as an extension of the main part of the foramen occipitale magnum.

The *occipital vertebral arch* is not quite so heavy and well marked in no. 886 as in Ia. The entire cartilage of the occipital region is somewhat lighter in structure. The lateral occipital eminence is not so prominent, nor is the cartilage here so thick.

The *condylar fossa* is represented upon the occipital cartilage by a depressed area, but as yet the superior articular process of the atlas does not lie far enough out to occupy it. It is relatively larger than that of the mature bone.

The *jugular process* is represented by a conspicuous transverse cartilaginous projection, which far overreaches the transverse process of the atlas below, in contrast to the condition in the adult.

There is a well-marked, though small, *processus ascendens*. It is a spheroidal mass of cartilage of rather young type which projects upward in the midline from the upper border of the tectum posterius—the central part of the supra-occipital cartilage. It is attached by a very short cartilaginous pedicle to the upper margin of the tectum. To either side, and closely associated with it, are the paired osseous spicules which represent the young interparietal bone, in their thin investment of condensed mesenchyme. The ascending process is thus attached to the unchondrified upper edge of the supra-occipital cartilage, already referred to, and which has been called by Bolk ('04) the 'Knorpelspange,' and other names.

Fawcett, in 1910, described this process in a human embryo of 30 mm., and homologized it with the *processus ascendens* of

the reptiles. This homology seems to me to be quite in order, but I would point out that the process in mammals does not bear the same relationship to the saccus endolymphaticus that it does in reptiles. Gaupp ('00) and Rice ('20) have described the saccus in lizards as lying just lateral to the ascending process, and Rice regards the process in the skink as affording protection to the saccus. In mammals, it need hardly be said, the saccus is far distant from this region, having accompanied the otic capsule in its evolutionary downward and outward rotation, and it follows that such a function on the part of the ascending process, if it ever existed, has become obsolete.

Mead, in 1909, made the same homologization for a small free nodule which he found in the pig's skull, just above the tectum posterius. In 1914 I described a free nodule of cartilage (with a very small grain of cartilage beside it) in the skull of Ia, and looked upon it as possibly representing the most superior cartilaginous mass described by Bolk ('04) in human skulls of about this stage and a little older. On account of the fact that the sections immediately posterior were missing from the specimen, I was unable to determine its relationship to the upper edge of the tectum posterius. It seems possible that these free cartilaginous masses may be vestiges of the tectum cranii anterius, a band which connects the parietal plates dorsally. Although this tectum is generally looked upon as rudimentary in man, Kernan ('16) has reported it as complete in his 20-mm. human embryo. Kernan has discussed the subject of the two tecta, lending support to the view of Levi ('00) that the anterior tectum disappears as the posterior appears. An interesting finding is reported by Fawcett ('18 b) in the embryonic skull of Weddell's seal. Two cartilaginous masses, lying side by side and showing slight signs of fusion with one another, appeared well forward in the membranous cranial vault, and are thought by Fawcett to belong to the anterior tectum.

I have found the ascending process in a number of embryos of the Carnegie collection, and am now engaged in a survey of this collection with the purpose of making a study of it. The process is represented in mesenchyme as early as the 21-mm. stage.

The work of Bolk ('04) upon the occipital region of human embryos has been much quoted in the literature, and has led a number of recent investigators into error because of a misinterpretation which it contains. Bolk used the van Wihje method to study the cartilage of this region, but the dyestuff (methylene blue) did not stain the calcified area of the supra-occipital cartilage representing the ossification center of the supra-occipital bone. Since this area did not give the tinctorial reaction for cartilage, and since he did not check his work with sections, Bolk concluded erroneously that it was membrane. His later stages show distinctly that this region does become ossified. He attempts to explain the occurrence of this 'membrane' by saying that rapid growth of the brain in this region has interfered with the development of cartilage; he fails, however, to account for the development of the 'Knorpelspange,' a band of cartilage which, as I have shown, represents the upper edge of the supra-occipital cartilage which has remained uncalcified, and which consequently stains blue in Bolk's preparations. It seems obvious that if the growth of the brain interferes with the development of cartilage in the region of Bolk's 'membrane,' it should certainly do so in the case of the 'Knorpelspange.' As a matter of fact, cartilage develops in the supra-occipital region quite early. I have found it at the 21-mm. stage in a human embryo. Calcification in the center for the supra-occipital comes on quickly. It should be said for one recent investigator (Fawcett, '10 b) that he was skeptical of this work of Bolk, saying (p. 306): "I must confess the appearances in his figures scarcely explain what is seen in this cranium."

It would seem that the upper edge of the supra-occipital cartilage, judging from Bolk's work, as well as my own, remains cartilaginous for a considerable time, and it may be that thus growth of the band in width is promoted through proliferation of the cartilage and subsequent invasion of it by the process of calcification, just as expansion of any endochondral center of ossification is brought about.

Bolk found, too, the region of the apex of the superior occipital incisure remaining cartilaginous, as shown by the staining with

methylene blue. The same reason may underlie the persistence of this cartilaginous mass. In one case, at least, Bolk finds that this paired mass of cartilage agrees in position with the later developing bones of Kerckring. These masses of cartilage, which Bolk thinks are in membrane, are doubtless really connected above with the calcified cartilage of the supra-occipital, and correspond probably to the processus descendens, as described in no. 886.

The *otic capsule* is well developed, the cochlear part showing the youngest type of cartilage, in accordance with the familiar order of chondrification. The walls are thin. A large *massa angularis* is present. The spiral septum is forming, but is far from complete. The cavity of the capsule was modeled as a solid, and the membranous labyrinth was also modeled. The latter is almost fully differentiated, and occupies but a small fraction of the available space within the capsule. The foramina are all large, and the edges are for the most part thin and of young cartilage.

The *malleus* and *incus* are separated by membrane, but do not show a distinct joint cavity. The *crus breve* of the *incus* is connected with the otic capsule by a small area of young cartilage. There is a small fragment of bone developing in the perichondrium of Meckel's cartilage, which represents the processus Folianus of the malleus, or goniale. It is as yet connected with the malleus only by membrane. The *stapes* has the well-known ring form, and the arciform base bends in the membrane filling the vestibular window. This membrane resembles pre-cartilage, and is not everywhere sharply marked off from the stapes. Anteriorly the latter is joined to the otic capsule by a narrow junction of young cartilage.

There was no *supracochlear cartilage*, as in Ia.

The *perilymphatic foramen* is large and shows evidence of the development of a partition which will separate it into its future parts; there is a small anterior and a somewhat larger posterior intraperilymphatic process.

The *styloid process* is directly attached to the crista parotica of the otic capsule by cartilage, whereas in Ia it was free from cartilaginous union with the capsule.

The *mastoid process* is a small free nodule in 886, whereas in Ia it was connected by cartilage with the otic capsule.

The *parietal plates* are relatively larger than in Ia. They have not, as yet, begun to be overlapped by the parietal bones. They are marked off from the occipital cartilage upon the cranial surface by the occipitoparietal groove. The cartilage along this groove is thinner than that of the plates above and below. Dorsomedially the parietal plate and occipital cartilage are separated by a distinct occipitoparietal notch, which, however, is not quite so deep as in Ia. There were no isolated nodules of cartilage in the vicinity of the upper margins of the parietal plates, as in Ia.

The *body of the sphenoid* is large, stout, and unperforated. From its side the *alar process* projects, and from the outer end of this process the temporal wing depends. From the caudolateral end of the alar process, which here is knobbed, projects backward the conical *aliochlear commissure*, whose pointed caudal end is confluent with the cochlear part of the otic capsule just below the pole of the latter. This commissure was not present in Ia, being represented by a short process, projecting backward from the processus alaris. The commissure subsequently develops into the lingula. The alar process, as Lewis ('20) points out, is largely taken into the body of the mature sphenoid, the carotid sulcus appearing upon its upper surface. The *carotid foramen* is closed by the aliochlear commissure in 886, and is much larger than the internal carotid artery which traverses its outer corner. Its posterior boundary is in part formed by a projection from the basisphenoidal cartilage which forms a union with the cochlear portion of the otic capsule, and represents the posterior petrosal process of the adult bone.

The *temporal wing* shows a distinct lamina ascendens and a pterygoid process. The foramen rotundum is complete, but the medial border is as yet thin and composed of young cartilage.

The *medial pterygoid plate* is a thin strip of bone, to the lower end of which is attached the cartilaginous hamular process. The line of junction between the two is not well marked.

The *orbital wing*, larger than the temporal, is thin. Its posterior root is stout and rounded, but the anterior root is of precartilage, is very slender and is evidently just forming. From the caudal edge of the anterior root there passes back a slender strand of precartilage, the fundament of the *prechiasmatic commissure*, which was represented in Ia in cartilage, and was much stouter. This commissure cuts off the small prechiasmatic foramen from the larger optic foramen. The prechiasmatic foramen is relatively larger in 886 than in Ia. Contrary to my former statement (Macklin, '14), it may persist in adult skulls—at least in those of younger age. I have recently noted these foramina in the sphenoid of a young adult in the osteological collection of Johns Hopkins Medical School. In several older skulls it was not present.

From the edge of the presphenoid, just in front of the posterior root of the orbital wing, there projects outward the *ala hypochiasmatica*. According to Kernan ('16), this structure represents a separate center of chondrification for the *ala orbitalis*. In 886 it is of a young type of cartilage, edged with precartilage, and is noticeably less developed than in Ia.

Each orbital wing has a well-marked dorsolateral process, which turns upward a little, as well as outward and backward. With the *limbus sphenoidalis* these two points make an angle of 131° , contrasting with the average of the corresponding angles taken from four mature skulls, which was 150° . Thus there is a flattening out of the orbital wing with subsequent development, associated, no doubt, with the growth of the brain in this region.

The anterior part of the orbital wing is bordered medially by the orbitonasal fissure, but laterally it is continued forward into the spheno-ethmoidal cartilage, which is connected with the anterior part of the ectethmoid by the spheno-ethmoidal commissure.

The *nasal septum* is not so stout as that of Ia, particularly along the lower border. It presents no superior paraseptal cartilages, as in Ia. The *cartilages of Jacobson* are not so far developed as those of Ia, but the different parts can be made out in them. They are of young cartilage and precartilage, and

the medial plate is connected anteriorly with the ventrolateral process and with the nasal septum. They are situated considerably below the level of the *vomeronasal organ*.

The *vomeronasal organ* was modeled, and is fusiform in shape. It is connected with the nasal cavity through a developing duct in which a definite lumen has not yet formed. The main portion of the organ suggests a coiled duct, but the lumen is not definite. The septum, medial to the organ, is not hollowed out, as in Ia.

The *mucous membrane* covering the septum is fairly flat, and shows an indistinct wide and low elevation which runs almost parallel with the upper border.

The *ectethmoid* is a thin, irregularly shaped plate of cartilage, upon the inner surface of which are to be seen the representatives of the future conchae. The superior concha is as yet of precartilage, almost entirely, and is wide, low, and rather indefinite. The middle concha is well marked, though not so well as in Ia, and it does not show such a definite continuity caudally with the maxilloturbinate as in Ia.

In the middle meatus there is a long slender process which may represent the *uncinate process* of the adult. It is altogether of precartilage and corresponds to the cartilage of the middle meatus of Ia, where it was a small nodule of young cartilage attached by a pedicle of precartilage to the ectethmoidal wall. The region of its attachment corresponds outwardly to the location of the posterior maxillary process.

The *inferior concha* is the largest of the three, and is the lower portion of the ectethmoid which has been bent upward and inward. Anteriorly it slopes downward, but posteriorly it projects almost directly inward, and the inner edge, covered with young cartilage, turns downward. Anteriorly it is bounded by the posttransverse incisure. The medial extremity of the maxilla, underlying the paraseptal process, makes of the incisure a foramen, leading into the inferior nasal meatus. There is a small cavity representing the sphenothmoidal recess, and one representing the superior nasal meatus. Anteriorly the *agger nasi* is represented very indefinitely by a low eminence.

The roof of the nasal capsule is very incomplete, the cribriform plates having only begun to form, and their loci being represented by gaping foramina; posterior to these there is a fenestrated covering of young cartilage.

The outer wall presents posteriorly the familiar flattened planum antorbitale which bears, near the upper surface, a very small paraethmoidal process, directly attached to the wall. In Ia this was a free nodule. The anterior portion of the plate shows several eminences. The superior nasal prominence is low and makes no corresponding concavity upon the inner surface. The middle prominence, or Sakterwulst of Voit ('09), is very conspicuous. The inferior prominence is represented in the interior by a hollow, and is continued backward into the long and slender parasseptal process. Upon the lower edge of the prominence there is a small tubercle, the superior alar process which appears to be taken into the lateral crus of the greater alar cartilage in later stages, according to the researches of Remke ('13), as stated in the atlas of Peter ('13). The parasseptal process, too, is shown by the same investigator to become separated later on into fragments to form the lesser alar cartilages. This process represents part of the anterior transverse lamina of the lower forms, and it is of interest that the lesser alar cartilages of the nose are derived from it.

There is a distinct posterior nasal prominence which leads backward, outward and downward as a ridge to join with the sharp lower edge of the planum antorbitale. The process is partially cut off by a distinct cleft from the posterior maxillary process, which is edged with a young type of cartilage, and which shows projecting forward from it the very slender spicule of osseous tissues representing the lacrimal bone. Lateral to these structures is the nasolacrimal duct. There is a distinct club-shaped paranasal process, the narrow end being attached to the capsular wall, while the blunt anterior end, which also points downward, lies just lateral to the nasolacrimal duct. In Ia it was free. In subsequent ossification this region is apparently included in the lacrimal bone.

An interesting structure in 886 is the *anterior cupular process*. It is a very long and slender hook-like process of precartilage. It is directly attached to the projecting anterior margin of the ectethmoid which forms the front of the anterior naris. It is crescentic in shape, the concavity looking outward, and containing the epithelial plug of the anterior naris. At the summit of the curve the two processes are very close together, and they are also quite close to the nasal septum here. The posterior end of the process is very sharp and turns outward. Rehmke ('13), in a somewhat later stage, has found this process represented in cartilage (Peter, '13), and in the 275-mm. stage (sitting height) he has noted that it joins with the ventrolateral process to form the medial crus of the greater alar cartilage. It is of interest to find the medial crus represented so early in precartilage.

The *nasolacrimal duct* together with the nasolacrimal sac and the lacrimal ducts were all modeled. There is as yet no distinct lumen. The duct is quite slender and has a curved course. The sac is not at all a marked dilation. The lower end of the duct is applied closely to the epithelium of the inferior meatus (which is an unleft plate in this region), but does not as yet open into the nasal cavity.

The position of the lower end of the nasolacrimal duct is far behind that of the corresponding structure in the lower forms. As an example (taken from many), we may cite the condition in the cat (Terry, '17), where the duct courses forward, laterally to the anterior transverse lamina (represented in homo by the parasseptal process), and opens into the nose in front of this lamina through what is really an extension of the anterior naris. We have noted in Ia that there is no cartilaginous connection between the parasseptal process and the nasal septum, and the same condition obtains in 886, and it would seem that this structure, in man, does not develop. Thus it is not impossible that the retrogression of the lower end of the nasolacrimal duct has been associated with the disappearance of the anterior transverse lamina. When this lamina is removed there is no cartilaginous impediment to the caudal migration of the duct, and its

consequent shortening. The shortening of the duct, too, has doubtless been associated also with a shortening in the length of the nose.

The *notochord* was modeled in relief. Its course is very similar to that described by Huber ('12) for his embryo J, no. 47, 32 mm., shown in his figure 10. It presents varicosities in the region between the tip of the dens and the basioccipital cartilage. In traversing the basioccipital it runs through the anterior end of the preossification center, and is here much attenuated—indeed it shows a break in continuity just at its point of exit from the plate. It comes into close relationship with the pharyngeal bursa in the well-known manner, and here presents more varicosities. After traveling close to the pharyngeal epithelium for some distance, it again enters the cartilage of the basal plate to terminate in the basisphenoid just below the root of the dorsum sellae.

Several of the cranial nerves were modeled, including much of the V and VII, and some of the IX, X, XI and XII. The related ganglia, as the semilunar, geniculate, vestibular, cochlear, sphenopalatine, jugular or glossopharyngeal and vagus petrous and nodosum, were modeled. The internal carotid artery, auditory tube and tympanic cavity, and stapedius muscle and tendon are among other structures modeled. The relation of the skull to the brain and to the external form is shown in a profile reconstruction.

Membrane bones. The membrane bones are all present excepting the nasal. Some of them are very small, as the tympanic interparietal, lacrimal, goniale and medial pterygoid plate. The maxilla is of interest in that it presents a groove upon the palatine surface which leads up to the tip of the frontal process. This groove represents the incisive suture, separating the maxillary and the premaxillary elements, as recently described by Felber ('19); (abstract by Schultz, '20). The upper end of this suture is imperfect, representing the region latest to close.

The cartilaginous branchial arch skeleton was modeled. The *hyoid cartilage* has a lesser cornu of young cartilage connected by a membranous strand with the styloid process. There is a

perforation in the *thyroid cartilage* which is closed below by precartilage. The *cricoid cartilage* bears the small *arytenoid cartilages*, which are very small nodules of young cartilage enclosed in a thick layer of condensed mesenchyme which shows several of the characters of the adult cartilage, and which is directly continuous with the mesenchyme of the cricoid cartilage. The tracheal rings are rudimentary as yet.

A full description of this skull with illustrations will appear at an early date in the Contributions to Embryology (Vol. 10), published by the Carnegie Institution of Washington.

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Resumen por el autor, George B. Wislocki.

Nota sobre el comportamiento del azul tripan inyectado en el huevo de gallina en vías de desarrollo.

Una solución coloidal, el azul tripan, fué inyectada bajo precauciones asépticas en huevos durante el undécimo día de incubación. El pequeño orificio abierto en la cáscara, a través del cual se inyectó, fué cementado, volviendo a colocar el huevo en la incubadora. Después de dos días se abrió el huevo examinando el embrión. El colorante fué inyectado en la cámara aérea, saco vitelino, saco alantoideo, saco amniótico y en el mesodermo del alantoides. En cada una de estas regiones se comporta de modo diferente. La membrana de la cáscara que rodea a la cámara aérea le absorbe rápidamente. Escapa desde el saco vitelino a través de las células epiteliales endodérmicas que forran el saco interiormente, pasando al mesodermo subepitelial, donde es absorbido y almacenado por las células mononucleares que rodean a un plexo de vasos vitelinos.

Cuando se inyecta en el saco alantoideo el colorante no es absorbido. Cuando se inyecta en el saco amniótico tiñe al epitelio amniótico y también penetra en el tracto gastrointestinal del feto por la boca. Cuando se inyecta directamente en el mesodermo de la membrana fetal se difunde en la corriente sanguínea del feto tiñendo vitalmente al embrión.

NOTE ON THE BEHAVIOR OF TRYPAN BLUE INJECTED INTO THE DEVELOPING EGG OF THE HEN

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THREE FIGURES

Attempts have been made by several investigators to determine the fate of dyes injected into the egg and their effect upon the embryo, but the experiments, though yielding a few results, were very brief.

Zaretsky ('10) injected trypan blue, trypan red, neutral red, methylene blue, fluorescin and eosin into developing eggs, and obtained results of greatest importance with the first two. He injected 0.5 to 1 cc. of one of these dyes into the air chamber of the egg, and after a period of days noted a slight staining of the amniotic fluid, but never any staining of the embryo itself. In other instances, after injecting the dye into the outer wall of the allantois, he observed a faint staining of the entire embryo, fetal membranes, amniotic fluid, and albumin sac. He stated that of the tissues of the embryo the kidneys were the most deeply stained, indicating a rapid excretion of the dye by that channel. Granules of dye could not be identified microscopically in the tissues.

Gräper ('12) reported the staining of early chick embryos with neutral red, trypan blue, and trypan red, but nothing more than diffuse staining of membranes and embryo alike was observed.

The most valuable results following the injection of dyes into the developing chick so far reported are those obtained by Bakounine ('95). She injected indigo carmine intravenously into a series of chicks ranging in age from three to fifteen days; excretion of the dye by the tubules of the wolffian bodies was demonstrable in the entire series.

The present paper is a preliminary account of some observations upon the behavior of trypan blue injected into developing eggs. The experiments were undertaken in spite of the failure of previous investigators to make any significant observations with vital dyes in the chick. It was hoped that by injecting the dye into different regions of the egg, some knowledge concerning the functions of the fetal membranes, and possibly a successful vital staining of the embryo, might be obtained.

Chicks of eleven days' incubation were used. Trypan blue, which forms a colloidal sol, was made up in 1 per cent strength in sterile distilled water. The injections were made with a syringe bearing a 26-gauge needle, 1 inch in length, through a nick in the shell, the opening being no larger than necessary to admit the needle. The trypan blue solution (0.2 cc.) was injected into each egg, whereupon the needle was withdrawn and the tiny opening in the egg-shell sealed with a drop of melted paraffin. The eggs were then returned to the incubator to be opened at the end of forty-eight hours—the thirteenth day of incubation.

By this method, of course, it cannot be determined exactly at the time of injection into what region of the embryonic membranes the trypan blue has been injected. If, with a general knowledge of the orientation of the fetal membranes and embryo, a number of eggs are injected, each locality which it is desired to inject will be reached in a certain number of eggs. This is made plain by reference to the diagram shown in figure 1, which illustrates conditions in the egg about the eleventh day. Thus it will be seen that by stabbing a needle into the egg, with a knowledge of the orientation of the structures within, one will in many instances inject with precision the yolk sac, allantoic sac, amniotic sac, etc. Orientation is made easier if one remembers that, after the egg has lain undisturbed in the incubator for a few hours, the embryo floats to the upper surface; hence the amniotic sac is more readily injected from above, and the yolk-sac from the side or below. Suffice it to say that in a series of five dozen eggs, injected as described above and killed on the thirteenth day of incubation, all of the selected points have been successfully injected. A description of the findings in each of the groups follows.

Injection into the air chamber. The air chamber is a cleft between the two layers of the shell membrane. The membrane is composed of matted fibers of organic substance which cross one another in every direction. When trypan blue is injected into this chamber it is rapidly absorbed by the shell membrane, much as it would be by blotting paper, staining it a deep blue. The adjacent structures beneath the shell membrane, namely, the chorion and allantois, as well as the embryonic fluids, remain

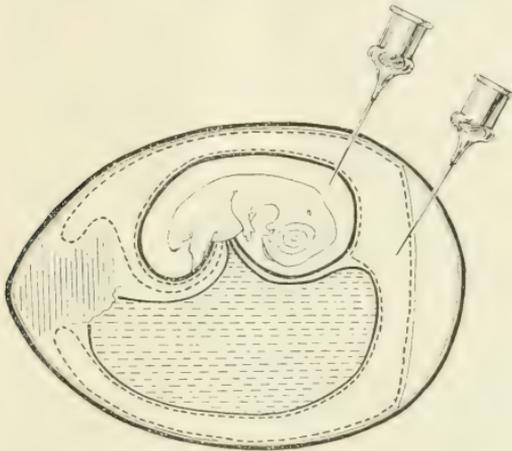


Fig. 1 Schematic representation of a chick approximately on the tenth day of incubation, showing the technique used in injecting different localities of the egg.

unstained and consequently also the embryo itself. When the shell membrane is removed, the inner surface of the shell is frequently found colored in the neighborhood of the air chamber.

Injection into the yolk-sac. On injecting trypan blue into the yolk-sac the dye remains somewhat localized in the yolk in the neighborhood of the injection, lending a greenish appearance to the yolk. The trypan blue, together with the yolk, is absorbed by the endodermal epithelial cells lining the interior of the yolk-sac. The dye imparts a greenish-blue coloration to the wall of the yolk-sac. Microscopically, a greenish, diffuse coloration

of the cytoplasm of the yolk-sac epithelium is visible. The dye enters the epithelium most abundantly in the region of the area vasculosa, where the wall of the yolk-sac is thrown into numerous folds covered by large columnar cells which possess swollen ends and the cytoplasm of which is closely filled with droplets of yellow substance.

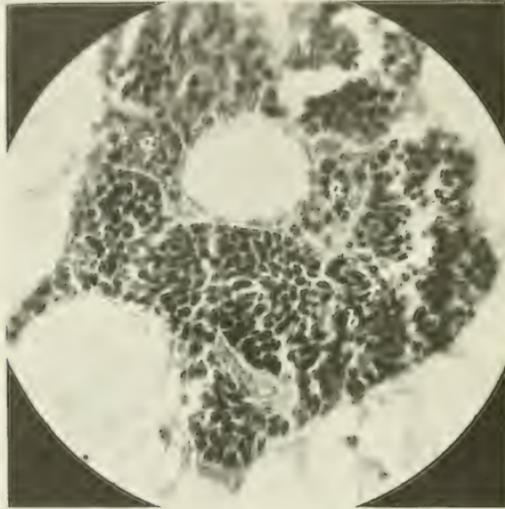


Fig. 2 Photomicrograph of the wall of the yolk-sac of a thirteen-day chick, showing a portion of the area vasculosa. A group of cells which ingest vital dye (a) is seen adjacent to a blood vessel. Other groups of cells (b) are hematopoietic and do not phagocytize dye.

The dye eventually penetrates the basement membrane upon which the endoderm rests and reaches its final destination in groups of cells surrounding the rich venous network in the yolk-sac wall. These cells are closely associated with groups of hemoblasts which invest the blood-vessels. The former are round or polygonal and possess a central or slightly eccentric round nucleus. Their cytoplasm absorbs the vital dye and stores it in the form of closely packed blue granules (fig. 2). These cells appear to be the ultimate destination

of the dye injected into the yolk-sac, as no evidence of its further passage is discoverable. The endothelial cells lining the blood-vessels remain unstained and, since the embryo itself does not become vitally stained, it is unlikely that any of the dye escapes into the vitelline vessels. It is of interest to note that in spite of the fact that throughout incubation the yolk-sac possesses a connection through its stalk with the intestine, none of the dye injected into the sac enters the intestine through this opening.

Injection into the allantoic sac. When trypan blue is injected into the allantoic sac it mixes uniformly with the allantoic fluid, coloring the latter dark blue. The dye does not escape from the allantoic sac—a fact which can be demonstrated in several ways. If the discolored allantoic fluid is drained off and the sac washed out with physiological salt solution, the allantoic membrane is found to be unstained. The fact that the embryo and all its membranes are unstained confirms the view that no dye has escaped from the allantoic sac. Furthermore, microscopic examination of the wall of the sac reveals the absence of the dye in the delicate, flat polygonal cells which cover its surface, or in the layer of star-shaped cells just beneath the surface. These observations accord well with the view that the allantois is a reservoir for the excretory products of the mesonephros and metanephros.

Injection into the amniotic sac. Trypan blue mixes uniformly with the amniotic fluid. The amniotic membrane becomes pale blue due to the presence of dye, which in some instances is visible under the microscope as blue dust in the delicate, flattened epithelial cells lining the surface of the membrane. The stomach and intestines invariably contain dark blue stained mucoid fluid which indicates that the amniotic fluid is swallowed by the embryo. Stained fluid is also observed in the lumen of the trachea and primary bronchi. A pale blue staining of the entire embryo is frequently observed, but the quantity of dye is insufficient to make it visible in the tissues when examined under the microscope.

Injection into the mesoblast of the allantois. When trypan blue is injected into the mesoblastic tissue uniting the allantois with the chorion and amnion, vital staining of the embryo results. This is due, no doubt, to the easy access which the dye has to the network of allantoic vessels through which it is soon conveyed to all parts of the embryo.

The membranes and integument of the vitally stained embryo present a light blue aspect. The yolk remains unstained, as does also the shell membrane and the shell itself. The depth of color in the various organs of the embryo proper varies: the central nervous system is unstained, the arteries are conspicuously blue, the lungs pale blue, the liver is greenish blue, the spleen reddish blue. The wolffian bodies are by far the most deeply stained organs; the metanephros is appreciably blue.

Microscopic examination of the tissues reveals the presence of trypan blue in granular form in several of the organs. In the thirteen-day-old chick it is found in most abundance in the wolffian bodies. Here it occurs in the shape of numerous tiny granules in the epithelium lining the uriniferous tubules (fig. 3). As might be expected from our knowledge of the distribution of vital dyes in the adult renal apparatus, no dye is discovered in the glomerular capsules. In the metanephric tubules, which at this period are already quite distinct, only traces of the dye are visible. It would appear, then, that at this period of development the wolffian body still serves as the main pathway of excretion, though regressive changes in the tubules are already plainly visible.

The second organ in which trypan blue appears in abundance is the embryonic liver. The dye is found microscopically in nearly all the endothelial cells lining the sinusoids and the terminal branches of the portal vein. In the liver cells themselves no particles are visible.

The spleen, which in the thirteen-day-old chick is a small round organ approximately 2 mm. in diameter, contains traces of trypan blue which appear to be within cells lining the vascular channels and occasionally within mononuclear cells lying free within the sinuses.

Nowhere else in the tissues of the embryo is trypan blue abundant, although traces of dye are encountered not infrequently in the connective tissue in cells resembling clasmatoocytes. That there are cells in the connective tissue of the chick capable of phagocytosis is best shown by examining the mesoderm at the site of injection of the trypan blue into the wall of the allantois.



Fig. 3. Photograph of a drawing of the wolffian body of a thirteen-day-old chick, showing a glomerulus with the beginning of a tubule. The black dots represent granules of vital dye.

Here numerous mononuclear cells, some of them round, others irregular in outline, are encountered with dye granules within their cytoplasm.

The behavior of trypan blue in the thirteen-day-old chick seems to be of sufficient interest to warrant an extension of these observations to other stages of development. The findings described suggest paths of investigation which it be might of interest to pursue. For instance, an investigation of the function of the mesonephros and the permanent kidneys as excretory organs

during embryonic life would be of considerable value. A study of the absorption of vital dyes from the yolk-sac at different stages would likewise furnish us valuable knowledge concerning the physiology of absorption in the embryo. The method used in these experiments will, however, never be applicable to chicks under four or five days. In these younger stages direct observation of the living developing chick under the microscope will always prove more satisfactory.

SUMMARY

A series of experiments is described upon the behavior of a vital dye, trypan blue, injected into the developing egg of the hen, and the technique of injection is given. In the vitally stained chick granules of dye are found in cells of the mesonephros, metanephros, liver and spleen.

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Resumen por el autor, H. E. Jordan.

Nota sobre la citología del cuerpo pineal de la oveja.

Las células de "interneuroglia" del cuerpo pineal de la oveja de cuatro a ocho meses se caracterizan, (en el tejido fijado durante cuatro semanas en una solución al 2 por ciento de ácido ósmico), por la abundancia de pequeñas mitocondrias esféricas y un número variable de glóbulos lipoides más grandes. Entre la cantidad de mitocondrias y la de gotitas de lipóide existe una relación recíproca bastante aparente. La existencia de una relación genética entre ambas estructuras, como se ha indicado, sigue siendo una cuestión pendiente de solución. Los glóbulos de lipóide constituyen la única prueba citológica indicadora de una actividad secretora. Las células carecen aparentemente de centrosoma y se dividen solamente por amitosis durante este período. Ciertas transformaciones, tanto fisiológicas como mecánicas, en las grandes gotas de lipóide producen estructuras que simulan un retículo de Golgi y un trofospongio.

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A NOTE ON THE CYTOLOGY OF THE PINEAL BODY OF THE SHEEP

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ONE PLATE (ELEVEN FIGURES)

In an earlier paper on the histogenesis of the sheep's pineal body, I ('11) stated the conclusion that 'There is no clear histologic evidence indicative of a glandular function. The sole cytoplasmic granules are melanic and probably have only ancestral significance' (p. 269). In a foot-note (p. 265) I recorded my experience with the Altmann osmic acid-potassium bichromate mixture, employed in an effort to reveal mitochondria. This technic disclosed the presence of very numerous deeply staining irregular granules. This granular character of the cytoplasm was interpreted as an artifact. But the Altmann technic preserved also certain large lipid globules of variable size, many of which were in process of disintegration into 'masses of very small black granules.' These lipid bodies I interpreted at the time as probably indicating 'intracellular degenerative changes, and not mitochondrial or secretory in nature.' My general conclusion was that 'If the pineal body of the sheep subserves an important physiologic function, this is probably active only during the first eight months of post-natal life' (p. 269).

Tilney and Warren ('19) conclude that the histologic evidence indicates that the pineal body of vertebrates should be classified as a glandular structure (p. 225). They state that 'The final conclusion to be drawn from the histological evidence in the epiphyseal complex of vertebrates would seem clearly to indicate that this structure of the pineal region possesses a pluripotentiality whose fundamental, inherent tendency is in the interest of glandular differentiation' (p. 226). They seek support, in part, for the conclusion of a glandular nature of the pineal body

from the occurrence of mitochondria in neuroglia. Granting that the cell constituency of the epiphysis is, in major part, neuroglia, as claimed by Dimitrova ('01) and by Jordan ('11), they state that 'this admission would not wholly invalidate the idea that the structure is glandular in nature, for, according to the most recent researches of Nageotte and Mawas, neuroglia cells contain mitochondria and hence, according to these investigators, should be considered as glandular elements' (p. 225).

Recently Portella ('20) has described and illustrated certain lipid bodies in the anterior lobe of the dog's hypophysis variously fixed and stained by methods designed for the demonstration of fat, which appear practically identical with those discernible in the epiphysis of the young sheep in tissue treated according to the Altmann technic. The presence of these lipid bodies he interprets as indicating a fatty secretion on the part of the hypophysis.

Mawas ('10) describes in the ependyma and general neuroglia of the central nervous system mitochondria, secretion grains ('grains de ségrégation'), and lipid inclusions. The mitochondria are variously designated 'chondriocotes' and 'filaments mitochondriaux.' The predominating type of mitochondrion seen by Mawas was apparently of the rod or bacillary type. The illustration given by Nageotte ('10) of neuroglia in the gray matter of the rabbit and guinea-pig, treated according to the Altmann technic, shows a small spheroidal type of mitochondria. Besides mitochondria, Nageotte describes also two other inclusions: 'grains vacuolisés' and 'grains de sécretion.' He claims that these three categories of granules are genetically related, the minute spherical mitochondria passing through a stage represented by a large granule with a clear center to a slightly smaller fuchsinophil secretion granule. These investigators base their interpretation of a secretory function on the part of the neuroglia, not upon the occurrence of mitochondria, as the quotation from Tilney and Warren would seem to suggest, but rather upon the presence of other cytoplasmic inclusions, namely, lipid globules and so-called secretion granules. The question of the genetic relationship between mitochondria and

the presecretion granules of glandular cells lies outside the scope of the present communication.

In my earlier paper I traced the histogenesis of the sheep's pineal from its origin from the ependyma to its definitive condition as a mass of essentially neuroglial constitution. During the earlier postnatal period two types of cells were distinguished: the so-called interneuroglia cells and the neuroglia cells. The latter represent simply more fully differentiated neuroglia elements. As compared with the generally polyhedral interneuroglia cells, the definitive neuroglia cells of the epiphysis are flattened, irregularly stellate or fusiform, with oval nucleus and smaller amount of cytoplasm. After the first year the interneuroglia cells become practically indistinguishable from the general mass of neuroglia tissue. The present investigation has reference only to the interneuroglia cells.

In view of the fact that mitochondria have been demonstrated in the neuroglia of the brain and cord, it would seem legitimate to infer that the younger neuroglia ('interneuroglia') cells of the pineal body also contain mitochondria. But I am not aware that anyone has actually described mitochondria in the cells of the pineal. However, the occurrence of mitochondria in the cells of the pineal body cannot be interpreted as indicative of a secretory function. Mitochondria have now been demonstrated in practically every kind of cell, non-secretory as well as secretory. In other words, the presence of mitochondria is not a specific index of secretory activity. Regarding the lipid bodies demonstrable by the Altmann technic, the case is different. The question remains whether these bodies, originally interpreted as a coincidence of the degenerative processes in the pineal, may not actually indicate a secretory rôle.

With these points chiefly in mind, I undertook a reinvestigation of the cytology of the sheep's pineal of the first year. I desired mainly to determine whether the finer cytoplasmic granulations of the cells as revealed by the Altmann technic might not be in fact modified mitochondria; and further, if possible, what is the relation of the mitochondria to the larger lipid bodies. But as the investigation progressed it involved

other cytologic details of the pineal cells, including thus, besides a consideration of mitochondria and the larger lipid bodies, also a search for evidence of a Golgi apparatus, a trophospongium, specific secretory granules, and a centrosome.

In a recent study of the cytology of the hemogenic giant-cells of the red bone-marrow of the rabbit and guinea-pig I ('21) succeeded in disclosing both mitochondria and a Golgi apparatus in the same cells by a slight modification of the Kopsch technic. I decided, therefore, to employ the same technic in a reinvestigation of the pineal body of the sheep. The following description is based chiefly upon material obtained from sheep ranging in age from four to eight months. Comparison with testicular tissue successfully fixed in the same way indicates that the fixation of the cells of the pineal is similarly satisfactory. The tissue has a generally clear golden-yellow color, with the cytoplasmic inclusions stained a deep brown or black. Unsuccessful preparations have a quite different appearance; either a straw color or a deep brown color, with a somewhat muddy appearance and a granulated character. The best-preserved area is some distance below the surface of the piece of tissue. The surface and center have generally the straw color and the muddy appearance, respectively, indicated above for poorly fixed tissue. It seems legitimate to infer that cytologic elements (except the centrosome) not revealed by this technic in this tissue do actually not occur; for this technic reveals mitochondria, Golgi net, and other lipid bodies in certain other tissues.

The tissue was obtained from the abattoir and fixed either within thirty minutes after slaughter of the animals, or after the heads had been on ice for from one to two hours. No essential differences were noted in the results following the two procedures. Pineal bodies of sheep of from four to eight months are essentially identical as regards the cytology of the interneuroglia cells. The distinctive melanic granules of the prenatal stages have generally disappeared by this period, and are nowhere sufficiently abundant to confuse the interpretation.

For purposes of comparison, portions of the same pineal body were in every case fixed also in a 10 per cent solution of formol,

in 80 per cent alcohol, and in the Zenker-formol mixture of Helly. The tissue was imbedded in paraffin, sectioned at from 3 to 5μ , and stained with hematoxylin and eosin. The Helly-fixed tissue was stained also with Wright's stain and with the Giemsa mixture. The latter procedures were used in an effort to disclose possible secretory granules other than lipid.

With none of the technics employed could a centrosome be disclosed. Nor could any cells be found in process of mitosis. Occasional cells occur with two nuclei, more or less closely apposed, indicating amitotic proliferation. In fetal and early post-natal life these cells divide at least largely by mitosis, as described in my earlier paper. It would seem that the later growth of the pineal body during the first year is largely dependent upon amitotic divisions. Lack of centrosome and amitosis may be causally related. In tissue prepared by the Kopsch technic a structure occasionally appears simulating a centrosphere with an included centrosome (fig. 4). This centrosphere simulacrum will be discussed below, where its deeply staining periphery, simulating a Golgi apparatus, will also be considered and interpreted.

Careful study of the preparations leaves little doubt concerning the presence of mitochondria in the pineal cells. They are generally of the granular, spheroidal type (figs. 1 and 2). Occasional bacillary and annular types occur. The former occur more generally in the more nearly spherical cells with a narrow shell of cytoplasm, apparently the younger cells; the latter occur mingled with the granular forms in the larger more irregular cells with a wide shell of cytoplasm, the more differentiated cells. Mitochondria are discernible in all of the cells, neuroglia as well as interneuroglia. The number of mitochondria varies greatly in different cells. They are generally relatively more abundant in the smaller polyhedral cells. There is also a fairly definite numerical correlation between the mitochondria and the lipid globules; when the globules are abundant the mitochondria are relatively few, and vice versa.

The lipid globules range in size from minute bodies to relatively enormous spheres (figs. 3, 6 and 7). The most difficult point in this connection concerns the line of demarcation between

a spherical mitochondrion and a small 'lipoid body.' This matter will be discussed after a further description of the larger lipoid bodies. The latter are apparently readily soluble, for they have disappeared in all of the specimens preserved in alcohol, formol, or Helly's fluid. They may occasionally be discerned as rather pale yellowish shadows in some of the cells in certain areas of tissue fixed with the strong solution of Flemming. Similarly, the smaller granules, presumably mitochondria, can occasionally be seen in cells of the Flemming-fixed tissue. The vacuolated condition of the cells of the pineal, fixed in other than osmic acid solutions, is undoubtedly largely the result of the solution of these lipoid constituents by the fluids employed in these technics.

The larger lipoid spheres undergo several distinct changes, all apparently, however, leading to the same end result, namely, liquefaction and disappearance. The large lipoid globules, as previously described for tissue treated with the Altmann technic, may fragment into a mass of minute spherical granules. More generally, in the tissue used in the present investigation (preserved according to the Kopsch technic), the globules become pale (dissolved) centrally and appear as more or less clear spheres with a deeply staining periphery (fig. 10). The latter represents the more resistant lipoid hull of the globule. This may be more or less regular, simulating a small Golgi apparatus (figs. 4 and 5). The interpretation of a Golgi apparatus is suggested especially when the clear central area contains one or several deeply staining granules, thus simulating a centrosphere with a centrosome. Almost as frequently the globules appear to dissolve peripherally, thus leading to a condition where a clear circular area includes centrally a deeply staining granule of variable size. Occasionally the globule dissolves in such manner as to have for a while both a deeply staining periphery and a central granule (fig. 9).

Frequently also one sees an entirely clear sphere surrounded by an envelope of small spherical granules, generally arranged in single file. The latter again suggests a localized Golgi apparatus in process of formation through fusion of mitochondria (figs. 5 and 8). A confident interpretation of these varying

conditions is difficult. As stated above, where the large lipid bodies are numerous, the small spherical granules (mitochondria) are relatively infrequent. The condition of the clear sphere with peripheral granules appears to represent two different things: In one case it represents a lipid globule which has fragmented into small granules, all of which have disappeared except the more peripheral ones. More generally, however, I believe that it represents a large globule which has become liquefied (or at least chemically changed so as to lose its former reaction to osmic acid) and has, during the enlargement involved in its growth or liquefaction, compelled a mechanical arrangement of the neighboring granules about its periphery. No doubt also such arrangement under pressure may subsequently effect a coalescence of the involved mitochondria and produce a clear sphere enveloped by a dark shell, thus again giving the appearance somewhat of a small Golgi apparatus.

Other than the complex above described as simulating a Golgi apparatus, there is no indication of such a structure in my preparations. Assuming that the interpretation as a Golgi-net simulacrum is correct, and that the Kopsch technic is specific for the Golgi net, we must conclude that the cells of the pineal of postnatal stages lack a genuine Golgi apparatus. Recalling that the Golgi net commonly forms first in the neighborhood of the centrosphere, the absence of a Golgi net in these cells may be correlated with the absence of the centrosome and the consequent amitotic mode of proliferation.

The cells of the pineal, whether fixed with alcohol, formol, Helly's fluid, Flemming's fluid, Altmann's fluid, or the Kopsch fluid, have a vacuolated cytoplasm. This vacuolization is least extensive in tissue fixed by the Kopsch technic. What vacuoles appear should probably be interpreted as the negatives of functionally liquefying globules. Such interpretation is correct no doubt also with regard to some of the vacuoles in tissue fixed with the Flemming and Altmann fluids. Some of the vacuoles no doubt are the negatives of globules dissolved by the fluids employed in these technics. The latter interpretation applies almost exclusively for the other technics. The vacuolization is

most extensive in alcohol-fixed tissue. Aside from the vacuoles, the cytoplasm of the cells preserved in 10 per cent formol is most nearly homogeneous. None of the technics employed disclose a specific granulation other than the mitochondria and the lipid bodies.

This brings us to the question of the relationship between the smaller spherical granules, interpreted as mitochondria, and the larger lipid bodies. Four obvious possibilities present themselves: 1) that the mitochondria become transformed into the larger globules; 2) that the mitochondria are derived by fragmentation from the larger lipid spherules; 3) that the mitochondria and lipid spherules sustain no genetic relation to each other, and, 4) that the entire granulation, including the most minute as well as the largest lipid bodies, are the same thing at different stages of growth, that is to say, that all the deeply colored bodies in the Kopsch-preserved tissue are either all mitochondria or all lipid bodies of different sizes. My preparations would seem to supply data for the partial support of any one of these interpretations. For example, the fact that one can find no line of demarcation, either on the basis of size or staining reaction to osmic acid, between the assumed mitochondria and the larger lipid bodies, might be used to support either the conclusion that they are one and the same thing or that the latter developed from the former. Furthermore, the reciprocal numerical relationship between the mitochondria and the lipid globules could be cited in support of the origin of lipid bodies from mitochondria, or vice versa. The fact that the larger lipid globules break up into smaller granules, indistinguishable from the assumed granular mitochondria, could also be used to support the contention that mitochondria are derived from lipid globules. And, finally, the mechanically imposed orderly arrangement of the smaller granules (mitochondria) about the growing and liquefying large clear spherules could be cited to sustain the argument of a relative independence of these two cytoplasmic constituents.

Similar difficulties have presented themselves in numerous other studies dealing with mitochondria and fatty globules.

Positive statements regarding a mitochondrial origin of fat are made, for example, by Schreiner and by Russo. Schreiner ('15) concludes that in the lipoblastic mesenchyme cells of the *Myxine* embryo fat globules are transformed mitochondria. Russo ('09) claims to be able to increase the mitochondrial content, and secondarily the deutoplasmic content, of the ova of rabbits by feeding or injecting lecithin. He believes that mitochondria are transformed into yolk globules. Coghill ('15), on the contrary, thinks he can see in the living tissue, and in sections, of amphibian embryos a granular transformation of the surface layer of certain deutoplasmic globules ('alpha globules') into mitochondria. The two elements, 'alpha bodies' of yolk globules and mitochondria, are said to react identically to certain assumed specific stains for mitochondria (e.g., janus green and Bensley's acetic-osmic-bichromate method).

The chemical relationship between mitochondria and certain fatty bodies is undoubtedly very close. Cowdry ('18) also has called attention to a general 'distinct reciprocal relationship between the amount of mitochondria and the amount of fat. Where there are few mitochondria there is much fat, and *vice versa*' (p. 82). At another place Cowdry says that '*Large spherical* mitochondria are usually on the border-line between true mitochondria and lipid droplets. They represent a stage in the transformation and may be found in any tissue where the change is taking place' (p. 69).

The tissue under investigation does not seem to me sufficiently favorable as a basis for a profitable further discussion of the question of the relationship between mitochondria and lipid globules. The only thing that can be said positively regarding this matter with the data at hand is that in the so-called inter-neuroglia cells of the pineal body of the sheep of the first six months there occur, as revealed by the Kopsch and Altmann technics, small bacillary and spherical granules similar to mitochondria of other tissues, and that among these occur large lipid droplets, and that the two are reciprocally related in amount.

Apparently the two elements sustain a close genetic relationship, but the proof of such relation is far from complete. It

may be emphasized, however, that neither of these structures can be interpreted as an artifact. In the first place, the fixation, as compared with other tissues similarly treated and where both mitochondria and Golgi apparatus appear sharply, seems perfect. Furthermore, the spherical vacuoles of the alcohol-fixed tissue are of the same relative size and abundance as the globules of the Kopsch-fixed tissue, indicating that the former are the negatives of the latter after solution by alcohol, and contradicting the possible argument that the lipid droplets of the osmic-acid-treated tissue are the product of a precipitation of this acid in this tissue.

The presence of vacuoles in the cytoplasm of these cells must be considered from the standpoint of a possible relation to a trophospongium. In the alcohol-fixed tissue the cytoplasm occasionally appears channeled by a system of irregular canals. The same is true in lesser degree of certain cells in the Kopsch-fixed tissue (fig. 11). Such a system of canals simulates the trophospongium of Holmgren. The appearance here must be interpreted, I believe, as the result of a partial coalescence and distortion of the vacuoles left after either the fixation-solution or a physiologic liquefaction and elimination of the lipid bodies of certain of these cells.

The results of this investigation of the cytology of the pineal body of the young sheep suggest the conclusions that these cells lack a centrosome, that they proliferate amitotically, that they lack both a genuine Golgi apparatus and a trophospongium, and that they are characterized by an abundance of granular mitochondria and a variable number of larger lipid globules. In the younger cells certain mitochondria are bacillary in type; in the later stages hollow-sphere types or ring forms occur. There is no definite line of demarcation, either dimensional or tinctorial, in Kopsch-fixed tissue, separating the mitochondria from the smaller spherical lipid droplets. The two sustain to each other a fairly definite reciprocal numerical relationship. Whether there prevails also a genetic relation remains undetermined. Other than the presence of lipid spherules, there is no indication in these cells of a secretory function.

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PLATE I

EXPLANATION OF FIGURES

The figures were drawn with the aid of an Abbe camera lucida, a 1-16 Leitz oil-immersion objective, and a no. 10 ocular. The magnification is approximately 1500 diameters. The cells are all of the 'interneuroglia' type, from the pineal body of a four-month-old sheep. The tissue was fixed for four weeks in a 2 per cent solution of osmic acid. The cells selected for illustration represent types of large groups of cells including many minor variations.

1 and 2 Less differentiated cells. The cytoplasm is crowded with mitochondria, predominantly granular, but including bacillary and ring forms.

3 Cell with spheroidal granules on the border-line between mitochondria and larger lipid globules.

4 Cell with granular and annular mitochondria; and a structure (at lower pole of nucleus) simulating a small Golgi net enveloping a centrosphere. The structure is actually a liquefying and dissolving large lipid body.

5 Cell with several similar structures, simulating Golgi nets.

6 Cell containing lipid bodies which grade without sharp line of demarcation from 'mitochondria' into larger lipid globules.

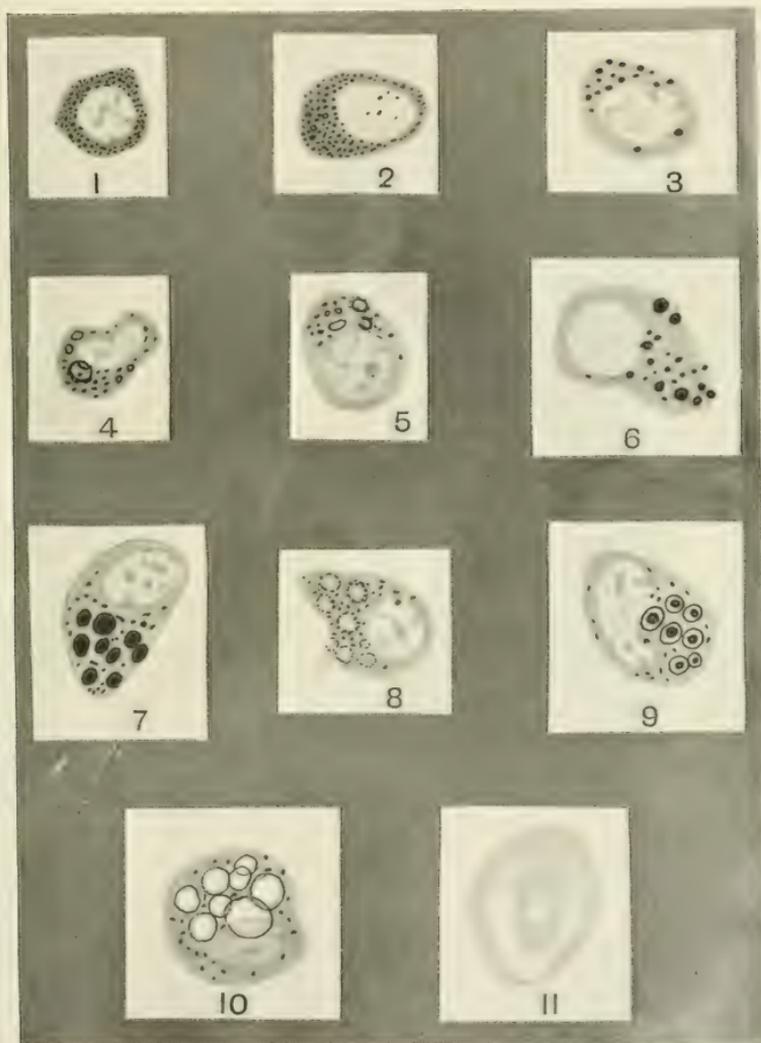
7 Cell with relatively many large lipid bodies and few mitochondria.

8 Similar cell at later (physiologic?) stage where the large lipid bodies have become liquefied and have lost their deep brown color, coincidentally effecting an arrangement of the granular mitochondria about their periphery in the form of a granular envelope.

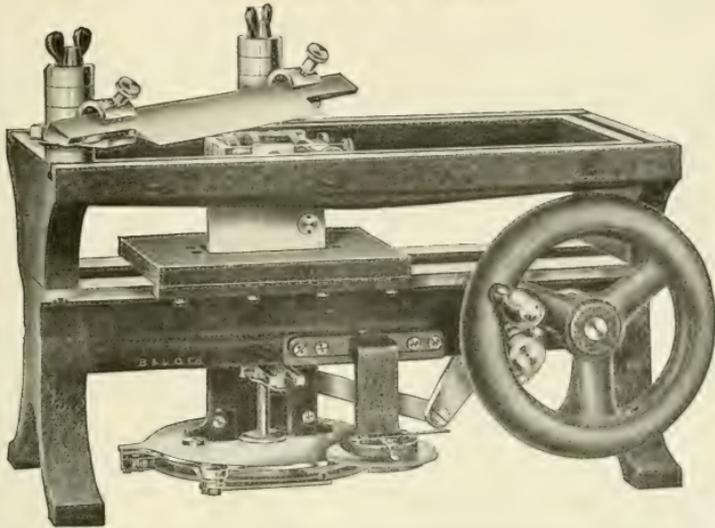
9 Cell in which the lipid bodies are undergoing solution, giving the appearance transiently of a clear sphere with a thin deeply staining hull and a central granule.

10 Cell at later stage, when the large lipid bodies have become almost completely liquefied.

11 Cell in which the liquefied lipid bodies (or their negatives) have coalesced, producing thus a canalicular formation simulating a trophospongium. The section passed outside the location of the nucleus.



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Resumen por el autor, E. V. Cowdry

Células flageladas en la tiroides del perro de mar (*Mustelus canis*).

Las preparaciones de la glándula tiroides del perro de mar teñidas con la modificación del método argéntico de Cajal introducida por Da Fano revelan el hecho de que cada célula está provista de un largo flagelo que se extiende dentro de la substancia coloidal. Puesto que Ferguson y otros han demostrado que los folículos son cavidades cerradas, esta flagelación de las células de la tiroide parece ser una interesante combinación de funciones motriz y secretora sin un valor adaptativo aparente. Puede sin embargo ser de algún interés en relación con el problema del origen de la glándula, indicando tal vez su desarrollo a expensas de un epitelio flagelado semejante al endostilo de las ascidias. Demuestra que la dirección primaria de la secreción es hacia la cavidad del folículo, como ha indicado Bensley, no hacia los vasos periféricos conforme supone Norris.

Translation by José F. Nonidez
Cornell Medical College, New York

FLAGELLATED THYROID CELLS IN THE DOGFISH (MUSTELUS CANIS)

E. V. COWDRY

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the Marine Biological Laboratory, Woods Hole, Massachusetts*

ONE PLATE (TWELVE FIGURES)

Preparations of the thyroid gland of the dogfish, made by Da Fano's ('20, p. 157) modification of Cajal's silver method, reveal the fact that each follicular cell is provided with a large flagellum which extends into the colloid substance. The flagella are blackened and show up distinctly in a golden-yellow background (figs. 1, 2, 4, and 5). The reaction is specific and calls to mind Levaditi's silver method for the staining of *Treponema pallidum*. In both cases there is a preliminary fixation in a formalin mixture followed by silver impregnation and reduction. The chief difference lies in the fact that Levaditi's method is designed for smears instead of for sectioned material. My best preparations of flagella have been impregnated for from four to eight days, which is too long for the satisfactory demonstration of the reticular apparatus. Having once studied them by this method, it is not difficult to recognize them in fragments of thyroid fixed in the usual way (formalin-Zenker, formalin-bichromate-sublimate, etc.) and stained with iron hematoxylin (figs. 3, 6 and 9).

Special methods like those of Loeffler and Bowhill, devised for the coloration of flagella in bacterial smears and depending upon the selective mordanting action of tannin, are not helpful. I have been unable to observe the flagella in living thyroid follicles teased out in sea-water, even after the slow addition of iodine, tannin, magenta, fuchsin, and other reagents which usually bring them to light in protozoa; neither have I been able to distinguish them with the aid of the new Leitz dark-field microscopic stage apparatus. It is possible that they are masked

by the colloid substance in which they are imbedded. The difficulty of trying to analyze a tissue several cells in thickness in which, when viewed with oblique illumination, there are so many high lights, has prevented me from obtaining decisive results. Isolated thyroid cells give but little information, because, having suffered so much from traumatism, they lose their characteristic shape and cannot be either identified or oriented with certainty.

Using Minchin's ('12, p. 53) criteria of distinction, I have called the filaments 'flagella' rather than 'cilia,' because of their relatively large size, because only one is provided for each cell, and, lastly, on account of their characteristic shape suggestive of free movement in all directions. As seen in the silver and iron-hematoxylin preparations, they present all the morphological characteristics of typical and permanent motor mechanisms. It will be noted that their organization is far more complicated than that of the transitory pseudopodia which Kite described some years ago in erythrocytes.

In silver preparations they sometimes appear to be beaded, as illustrated particularly in the uppermost cell in figure 1; but staining with iron hematoxylin shows that they possess, in reality, smooth and even outlines (figs. 3 and 6). The portion lying in the colloid substance is of even diameter and does not taper noticeably at the end. The fact that its shape varies in different thyroids, as well as in individual follicles of the same gland, seems to indicate different degrees of physiologic activity. Contrast the gentle curves seen in figures 3 and 6 with the typical spirals shown in figure 5. I have been unable to distinguish between the contractile axial filament and its ectoplasmic investment in the free portion of the flagellum, but in specimens fixed in formalin-Zenker and stained with iron hematoxylin the cytoplasmic part is shown with diagrammatic clearness (fig. 3). The flagellum is inserted about the center of the follicular margin of the cell, where, after penetrating through the cell membrane, the ectoplasmic sheath terminates in a small enlargement which stains intensely with iron hematoxylin or blackens with silver, depending upon the technique used. A very slender axial filament continues in the direction of the nucleus and ends in a typical blepharoplast.

The blepharoplasts are intensely blackened with silver, as one would expect from their centrosomal nature. They are also very closely associated, in a topographic sense, with the reticular material (figs. 4 and 7) which separates them from the nuclei. Unfortunately, the silver method which I have been using does not bring both to light simultaneously in the same thyroid cell. The living cells contain a large number of lipoid granules which may be supravitaly stained with neutral red. They may also be fixed in Bensley's ('10, p. 192) formalin-bichromate-sublimate mixture and stained with iron hematoxylin. In such preparations it will be seen that they are reduced in number in the immediate vicinity of the blepharoplasts (fig. 6). This reduction in cytoplasmic granulation is also a centrosomal characteristic.

The colloid substance is optically homogeneous, but may be differentially stained by the addition of a little acid fuchsin to the sea-water in which the follicles are being studied. After a few minutes, it becomes slightly granular, but the granules are stationary, exhibiting no currents or eddies which one might expect flagellar action to produce.

We may infer that the flagella are not transitory structures which are thrown out and withdrawn by the follicular cells in different phases of secretory activity, because, as far as I can ascertain without cutting complete serial sections, all the cells of the gland, regardless of their physiologic condition, are provided with them. They are as well developed in swollen cuboidal cells as they are in flattened cells, though the latter are generally supposed to be less active functionally. This may be seen by comparing figures 1 and 2. Cells whose nuclei are displaced toward the follicular lumen through the heaping up of colloid substance in the cytoplasm near the peripheral blood vessels possess typical flagella which are in no way altered (fig. 8). In these 'colloid' cells the lipoidal granules are also pushed toward the lumen (fig. 9), but the reticular material retains its usual position (fig. 7). Neither do I believe that the flagella are restricted to certain individuals of the species, because I have repeatedly confirmed their existence in adult dogfish captured

and examined as follows: June 18th, 20th, 23rd, 26th, 29th, and August 23rd.

I have examined several other elasmobranchs to determine whether flagellation is a phenomenon of wide occurrence which has been overlooked because investigators have not been in search of it. Ordinary preparations do not reveal it unless they are examined with special care. Energetic coagulants cause a shrinkage of the colloid substance which tears the flagella away from the cells, leaving them with a perfectly smooth border. One dusky shark (*Carcharinus obscurus*) and one sting ray (*Dasyatis centura*) show no trace of flagellation, but flagella are undoubtedly present in some at least of the thyroid follicles of one summer skate (*Raia erinacea*). They are illustrated in figures 10, 11, and 12. In this single specimen I have been unable to detect typical blepharoplasts, though I strongly suspect their occurrence. Each cell seems to possess a single flagellum which, as in the dogfish, may be wavy or spiral in shape, suggestive of internal tension; but the thyroid is so aberrant in its general structure that I hesitate to draw any very definite conclusions on the basis of insufficient material which I have not had an opportunity to re-examine. The follicular lumina, in addition to being invaded by large numbers of leucocytes, as noted by Ferguson ('11, p. 206) in *Carcharias*, contain concretions of great variety as well as large isometric crystals which can be readily seen unstained in living follicles in sea-water. Moreover, the epithelium of individual follicles occasionally varies in height from low cuboidal to tall columnar cells. Some of the follicles communicate and branch. It is possible that further study of the dusky shark and the sting ray will likewise reveal flagellation.

The uniform flagellation of the entire thyroid in the dogfish is quite different from the ciliated cavities which are occasionally described in treatises on the pathologic anatomy of the human thyroid gland. The ciliated cavities are directly derived from the pharyngeal epithelium, do not contain typical colloid substance, and are not lined by true thyroid cells. Ferguson ('11, p. 191) has shown that in the dogfish the thyroid follicles are isolated structures with closed cavities which are not in any way

connected with the pharynx by a duct system. It is a simple matter to verify this independence by teasing a small fragment of tissue in a dilute solution of neutral red in sea-water which stains the lipid content of the follicular cells intensely and delimits them sharply.

Norris ('18, p. 216) has studied the development of the thyroid in a closely related form, *Squalus acanthias*, and finds that the thyroid, as in other vertebrates, is at first a solid outgrowth of the pharyngeal epithelium in which the follicular cavities develop secondarily. What, we ask, is the stimulus which calls forth the formation of a flagellum in each of these follicular cells? It is almost inconceivable that such an elaborate mechanism should not serve some useful purpose. Although I have not actually observed movement of the flagella, they have all the distinctive characters of a typical motor apparatus such as one meets with in the protozoa or in hydra.

A special mixer to stir up the follicular contents is perhaps less needed in the dogfish than in mammals, because the thyroid lies in the median line on the coracohyoideus muscle immediately posterior to the mandible and is covered by the constrictor pharyngis and coracomandibularis muscles, so that pressure is brought to bear upon it with each movement of the jaws. There are some unique features in the general physiology of the dogfish; but I am unable to find anything approaching a clue. In the absence of direct information from extirpation experiments, about all we can say is that, unlike the mammalian thyroid, the thyroid of the dogfish plays no part in the development of a bony skeleton, because no such skeleton is present in elasmobranchs. It has been suggested by F. D. Thompson that the thyroids of elasmobranchs may have to discharge some of the functions of the parathyroid glands which are not recognized below the amphibia. The absence of a physiologic reserve of glycogen in either the liver or muscles of the dogfish¹ suggests a marked difference in metabolism which may in some way be reflected in the thyroid gland. There is also a considerable amount of

¹ Personal communication from Dr. H. C. Bradley.

urea in the blood. But it seems incomprehensible that these factors should have any direct bearing on flagella formation.

While I do not venture to suggest that these thyroid cells have developed flagella merely because they acquired the habit years ago, a brief consideration of the ancestry of the gland may be helpful. According to the most widely accepted theory, originally advanced by W. Müller, the thyroid is considered to have developed in the course of evolution from some structure like the endostyle of ascidians. This endostyle is an epithelial groove in the ventral wall of the pharynx along which food is passed to the stomach. It is lined with several longitudinal rows of cells possessing stout cilia (or flagella) and also by mucous cells which become more numerous near the stomach. The gland cells produce a thick adhesive secretion in which minute microorganisms and other food particles are entangled and become rolled up by the action of the cilia into a rounded bolus of suitable size for digestion. The evidence for homology with the mammalian thyroid is not very direct. It consists of close correspondence in position and of strong collateral evidence that ascidian-like forms are in the line of our ancestry. It is, in our opinion, a little unsafe to postulate a close genetic relation between the mucous secretion of the endostyle and the mammalian colloid, because they are so different chemically and apparently serve different functions, but the flagellation, which I have found in the dogfish, may be of some phylogenetic significance.

It is interesting to note in this connection that Camp ('16, p. 406) has studied the development of the suprapericardial body in *Squalus acanthias* and finds that it "represents the ventral extremity of a rudimentary seventh gill pouch" and that it consists in part of closed follicles containing goblet cells which secrete mucus. While it has not been definitely shown that the suprapericardial bodies find their homologues in the lateral thyroids of mammals, their striking resemblance to thyroid tissue and the presence in them of mucous cells may have some bearing upon our problem.

In the same way that the flagellated thyroid cells of the dogfish may be distantly related to the ciliated (or flagellated?)

cells of the endostyle, so the mucous cells of the suprapericardial body in *Squalus acanthias* which develop from the pharyngeal wall farther back and nearer the stomach, may be related to the mucous cells of the endostyle which are said to be more abundant in its posterior part. It is possible, therefore, that we have represented in the thyroid and pericardial bodies of elasmobranchs all the known components of the endostyle of tunicates. Unfortunately, our actual knowledge of the minute structure of the endostyle is very scanty, because investigators seem to have contented themselves with the descriptions published by Fol, Müller and Dohrn, written about forty years ago without much regard for cytologic detail.

We may at least conclude that the presence of flagella extending from the cells into the follicular cavity indicates that this cavity originally communicated with the pharynx; in other words, that the flagella constitute definite clues to the primary polarity of the cells.

This homology of the follicular cavity with the lumen of the ancestral gland, whatever it may have been, is difficult to reconcile with Norris's ('18, p. 214) hypothesis. He finds that the thyroid exhibits intraglandular cavities which are quite independent of the original thyroid pouch at approximately the same stage in the development of *Squalus acanthias*, birds, and mammals. While admitting that any attempt to interpret "their immediate or general biologic significance, although interesting, can at best be only speculative," he suggests that they "appear in response to a tendency to reproduce the ancient lumen or duct of the ancestral gland." As development progresses, they open on the surface of the gland and become invaded by mesenchyme and blood vessels. The epithelial cells in their walls become disposed in two layers, between which the definitive follicular cavities develop so that in the adult the primary polarity of the cells bordering the follicles is (according to him) directed toward the peripheral blood vessels (which occupy his intraglandular spaces), and Bensley's conclusion "that the thyroid cell represents a true reversal of polarity" is

quite unnecessary. I am unwilling to accept this suggestion for two reasons:

In the first place, all digestive glands which develop through an evagination of the wall of the primitive alimentary tube seem to maintain their epithelial lining intact. We are asked to believe, in the case of the thyroid, that this epithelial investment breaks down completely and that blood vessels, lymphatics, nerves, and connective tissue grow into the intraglandular spaces, or primitive lumina—biologically speaking, that they migrate outside of the organism, for the duct was, we suppose, originally in direct communication with the outside environment. This is a process, to the best of my knowledge, without counterpart in the development of other glands.

In the second place, Norris's claim (p. 215) that these spaces are quite independent of the follicular cavities in *Squalus acanthias*, as well as in all representative vertebrates which he has studied, would point to the conclusion that they are also independent in the dogfish, which is a closely related form. Since we know by their flagellation that the follicular lumina must represent the ancestral duct, it follows that we must seek some other explanation for the intraglandular spaces of Norris.

We may, therefore, accept Bensley's conclusion that, when in the mammalian thyroid secretion passes directly into the blood vessels, instead of into the follicular lumen (ancestral duct), we are actually dealing with a true anatomic and physiologic reversal in polarity.

SUMMARY

The development of flagella in the thyroid gland of the dogfish is an interesting combination of motor and secretory functions without apparent adaptive value. It may, however, have some bearing upon the problem of the ancestry of the gland indicating perhaps its development from a flagellated epithelium like the endostyle of ascidians. It does show that the primary direction of secretion is toward the follicular lumen, as claimed by Bensley; not toward the peripheral blood vessels, as suggested by Norris.

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PLATE 1

EXPLANATION OF FIGURES

All the figures have been drawn with a Zeiss apochromatic 2-mm. objective, compensating ocular, no. 8 and camera lucida and are reproduced without reduction. Figures 1 to 9 are of the thyroid of the dogfish (*Mustelus canis*) and figures 10 to 12 of the skate (*Raja erinacea*).

1 Thyroid cells with large flagella blackened by the silver method. At the base of each flagellum a typical blepharoplast may be seen.

2 The same showing that the size of the flagellum is not decreased when the thyroid cells become flattened.

3 Thyroid cells fixed in formalin-Zenker and stained with iron hematoxylin. The lipid granules which are illustrated in figures 6 and 9 have been dissolved out so that the axial filaments of two of the flagella can easily be distinguished passing into the blepharoplasts.

4 Thyroid cells prepared by the silver method with the reticular material impregnated and the flagella slightly darkened. Although the blepharoplasts cannot be identified, the axial filaments probably terminate in close association with the reticular material.

5 Thyroid cells treated by the same method showing wave-like and corkscrew-shaped flagella. The flagella of different animals and sometimes of neighboring follicles in the same animal often show great variations in shape suggestive of different degrees of physiological activity.

6 Thyroid cells fixed in Bensley's formalin-bichromate sublimate mixture and stained with iron hematoxylin which preserves both the lipid granules and flagella. The granules are less abundant at the points of insertion of the flagella.

7 Group of cells prepared by the silver method, showing the gradual accumulation of colloid material in the zone of cytoplasm bordering the peripheral blood vessels with displacement of the nucleus toward the lumen. Since the position of the reticular material is unaltered, we are not dealing with a true reversal in polarity as described elsewhere (Cowdry, 1922) in the thyroid of the guinea-pig.

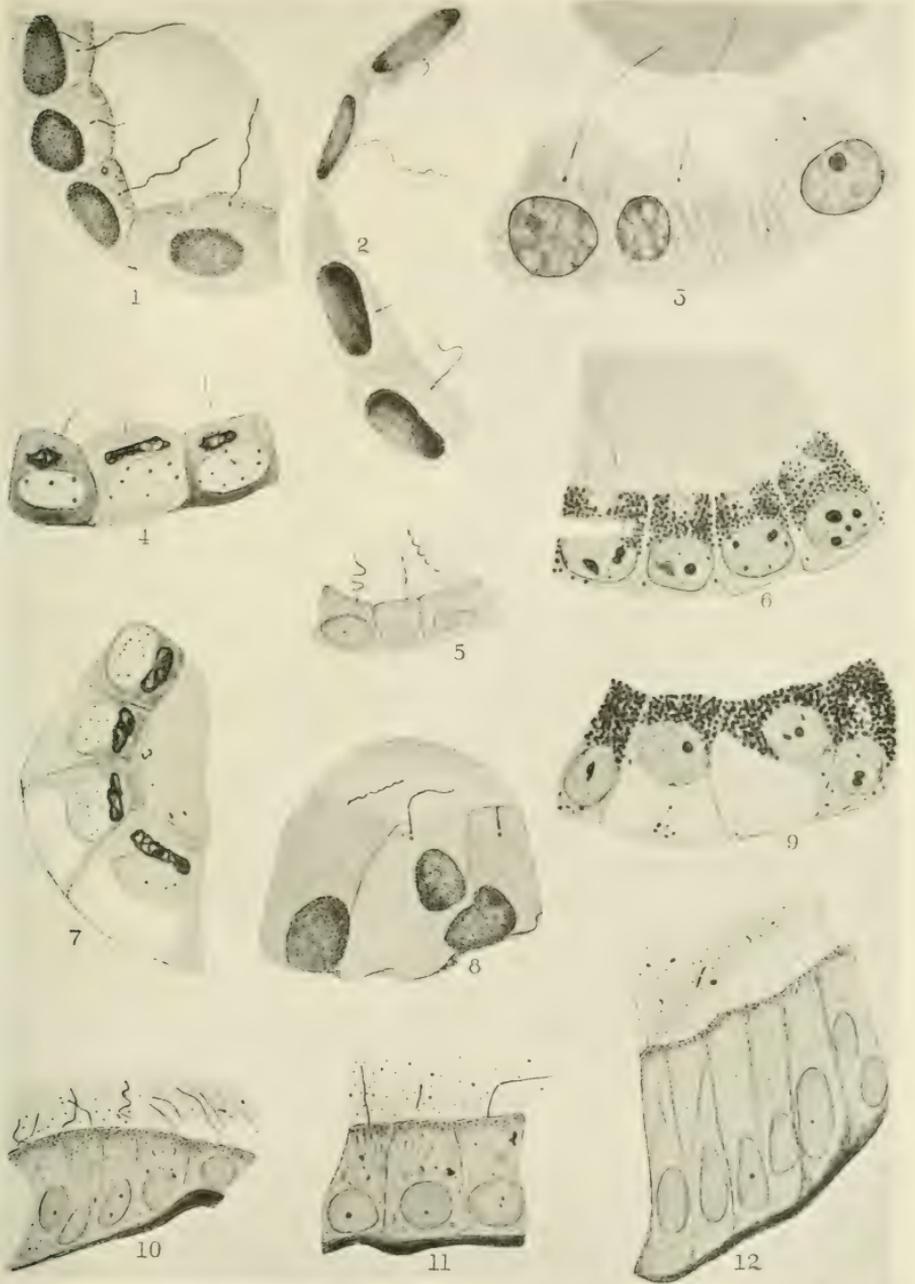
8 Thyroid cells prepared by the same method, but treated five days longer with silver which has blackened the flagella. In the colloid cell it will be seen that the flagellar apparatus is in no way modified, in spite of the fact that a large amount of colloid material has collected in the peripheral cytoplasm and the nucleus has been displaced towards the lumen.

9 Thyroid cells from the same tissue as figure 6, showing that the lipid granules remain unmodified in colloid cells. The flagella are unstained. A few rod-like mitochondria may be made out in the peripheral cytoplasm.

10 Thyroid cells of the skate prepared by the same silver nitrate method and bearing slightly undulating and spiral flagella extending into the colloid substance.

11 Cells from the same specimen each provided with a single flagellum. The blepharoplasts cannot be seen.

12 Group of flagellated, columnar cells also from the same specimen. Since the cells are so closely packed together and are not very distinctly outlined, it has been difficult to associate them with their respective flagella.



Resumen por el autor, Edmond Souchon

Un resumen de la conservación de las disecciones anatómicas con color permanente de los músculos, vasos y órganos.

El autor ha usado durante algún tiempo como solución permanente una solución compuesta de cloruro cálcico y formol, pero nuevos experimentos han demostrado que la sal cálcica no es necesaria. El agente activo es el formol. Una solución de 5 onzas de formol en un galón de agua clara conserva los tejidos e impide el enturbiamiento del líquido. El formol no decolora las disecciones preparadas por los métodos Souchon. La evolución de dichos métodos se basa en los siguientes hechos: Primero, que las disecciones de los músculos sumergidas en una solución de glicerina durante algunos días y después abandonadas al aire libre se oscurecerán mucho al cabo de cierto tiempo. Segundo, que el formol actúa como decolorante de los tejidos coloreados normalmente. Tercero, que las arterias y venas pintadas toleran la acción de cualquier solución ordinaria empleada para la conservación de los tejidos.

Translation by José F. Nonidez
Cornell Medical College, New York

PRESERVATION OF ANATOMIC DISSECTIONS WITH PERMANENT COLOR OF MUSCLES, VESSELS, AND ORGANS

A SUPPLEMENTARY NOTE

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The results of the experimental work on the preservation of anatomic dissections have been published in several articles in *The Anatomical Record* of Philadelphia and elsewhere.

During the evolution of this work it has undergone a number of changes, alterations, substitutions, and additions, etc.

I here publish a supplementary note presenting the final status of the methods.

With regard to the curing method, at the end of three days the preparation is taken out of the glycerin and exposed to the air in a room until the muscles become of a marked brown color. The longer the exposure to the air, the darker the brown. The darker the brown, the darker will be the final color of the muscles in the solution. The shade of the final color can be graded in this way. This curing method has superseded the chemical method.

It is then placed in a permanent solution. For a time I used a solution composed of calcium chloride and formol, but further experimentation has shown that the calcium is not necessary. It is the formol that is the active agent.

A solution of 5 ounces of formol to 1 gallon of clear water will preserve and prevent cloudiness. The formol must be as clear as water. If greenish, it will discolor the solution. If greenish, it should be filtered through bone-black before using to make it clear. Before using the solution, filter it anyhow through bone-black and paper to obtain filtered clearness.

It may be that 3 ounces of formol may do. Experimentation will determine that. The formol will not bleach the dissections prepared by the Souchon method.

THE EVOLUTION OF THE SOUCHON METHOD FOR THE PRESERVATION OF ANATOMIC DISSECTIONS¹

I have always been very much interested in the various phases through which discoveries and inventions went before reaching the goal. For this reason I am giving here the various stages of the Souchon methods.

The two methods rest on three facts, none of which is new separately, but is new in the application or combination. They are: First, that dissections of muscles immersed in solution of glycerin for a few days and then removed and left in the open air or enclosed in a receptacle will become very black in coming time. Second, that formol was a bleacher of colored tissues. Third, that painted arteries and veins would stand the action of any ordinary solution used to preserve the tissues. Although I knew all these facts years ago, it took me ten years of work and experiment to bring together the combinations that brought about the results.

When I found out that glycerin would blacken the muscles, I tried several chemicals to reduce the blackness, and it is only after several years of experimentation that I struck formol, which did the work.

I had used many years ago solutions of gelatin to paint pastel drawings before trying a thin coat of damar varnish, but it was only after two or three years of unsuccessful experiments at painting muscles that I bethought myself of first applying a coat of gelatin to the muscles. That gave success.

The elimination of the calcium chloride from the calcium formol solution is due to the fact that I always follow the rule of Pasteur, which consists, when a work is completed, to go over it critically, changing, etc., with a view of simplifying it.

¹ At the New Orleans Meeting of the American Medical Association in 1920, the Committee awarded to Dr. Edmond Souchon the Gold Medal for the best scientific exhibit of his anatomic dissections. The dissections were made according to the Souchon methods.

The only real new discovery was that solutions of 5 per cent formol would not in time bleach the dissections prepared by the Souchon method. They will bleach dissections prepared by other methods.

Resumen por el autor, Wesley M. Baldwin

La producción artificial de siringomiocele en el renacuajo por medio de los rayos X.

El autor ha sometido los huevos de la rana toto, rana verde, rana leopardo y sapo común a la acción de los rayos X emanados de un tubo especial de aire frío de Coolidge. Veinticinco de los huevos de la rana toro en una serie de 100 presentan en secciones seriadas una verdadera condición de siringomiocele. Los defectos notados en los cortes comprenden un desarrollo anormal del corazón, vasos sanguíneos y pericardio dilatados, una circulación estática y una marcada dilatación del neurocele en los niveles segmentarios que indican la zona de unión del cuerpo con la cola.

La dilatación del neurocele está acompañada por un pronunciado adelgazamiento de la pared dorsal del tubo neural, por la posición lateral de los miotomos y por la ausencia de la fusión de las mitades del arco neural.

Translation by José F. Nonidez
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THE ARTIFICIAL PRODUCTION OF SYRINGOMYELO- CELE IN THE TADPOLE BY MEANS OF X-RAYS

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At the time of the consideration of "The artificial production of monsters demonstrating the condition of spina bifida as the result of the action of ultra-violet light rays," as published in the *Anatomical Record*, vol. 9, no. 9, May, 1915, the several differences in anatomic structure, differentiating this condition from that group of the same name appearing occasionally in the human embryo, were appreciated. Subsequently, an effort was made to approximate more closely the human condition through the utilization of the same physical agent, but with a variation in the method of application.

It has been ascertained since the publication of the paper on "The artificial production of monsters conforming to a definite type by means of x-rays," *Anatomical Record*, vol. 17, no. 3, November, 1919, that x-ray energy was fully as dependent a physical agent in the production of spina bifida as ultra-violet light ray energy. The mechanics of production appeared to be identical with that reported previously. Certainly, the end-product differed in no apparent histologic detail from that produced by the light rays. As was the case in the instance of the latter, the causative factors were resolvable into a single inhibitive force preventing the medianward migration and fusion of the hemineural tubes.

On the other hand, were the morphologic features the only details to be considered, one might assume, in making a comparison between the spina bifida of human monsters and the spina bifida resulting from experimental methods, that the former condition, in contrast to the tadpole, reflected a com-

paratively complex, causative agency. Manifestly to a single inhibitive force could be referred neither the thinning of both the epidermis and of the sublying mesenchymal tissue, the separation and defective development of the myotome halves, nor the bulging of the cord through a dilatation of its canal or a protrusion of the meninges. It might be assumed, however, that the interposition of an inhibitive force between the neural-arch halves, thereby inducing a separation and lack of coalescence of them, together with a concomitant cleavage of the overlying myotome halves, might permit the cord and its meninges, by virtue of this resultant median cleft, to approach more or less intimately into contact with the overlying epidermis. There would still be lacking under these conditions, however, an explanation of the myelocoelous condition and of the bulging of the meninges. It would appear at first consideration that these latter factors were rather the resultant of a generalized force acting upon the vascular system as a whole including the lymphatic system, with possibly a sharply circumscribed defect of the neural tube.

Incidentally, such defects have been noted previously and reported in the earlier papers as accompanying marked morphologic defects of the heart. Such have been characterized by dilatation of the neurocele and thinning of the dorsal parietes of the neural tube. The superabundant cerebrospinal fluid, together with the bulging membranes of the cord, and the dilated neurocele seemed altogether to point directly to a generalized defective condition of the vascular system, most probably localized in part, at least, in the heart, which had induced a stasis of circulation.

In several of the specimens reported in the paper of November, 1919, it was noted that the expanded encephalocele with its thinned dorsal parietes occasionally featured the neurocele as well and, singularly enough, at a most usual level at the junction of the trunk and tail. The expanded pericardium, together with the dilated lymph spaces under the ectoderm, furnished contributory evidence upon the essential causative factor, the imperfect development of the heart. If these inferences were correctly

drawn, therefore, the agencies underlying the condition of human spina bifida must be referable to, at least, two separate and independent group factors—one responsible for the separation of the neural arch-halves and of the myomeres, and the other for the dilatation of the neurocele and the consequent thinning of its dorsal wall together with the bulging of the meninges.

The author has thus far succeeded in producing one type of spina bifida, resembling, in every histologic detail, syringomyelocele in the human. The experimental production of this condition cannot be controlled at present, however, with the same degree of certainty as was true of spina bifida of the tadpole. The developmental agencies which underlie the defect have been recognized as the two which are enumerated above. The causative physical agent employed was x-ray energy.

The eggs used for the experiment were those of the bullfrog. These were obtained and rayed during the one- and two-cell stages. A general exposure of the egg for two minutes was given at a distance of 4.8 cm. from the center of the target. The apparatus consisted of a special Coolidge air-cooled x-ray tube, such as was described in the *Anatomical Record* of November, 1919. Upwards of 100 eggs were used in the study, twenty-five of which showed, upon careful study of the serial sections, the condition of syringomyelocele. These were the optimum results with 100 eggs. Unfortunately, it has not been possible to increase the percentage of production of this anomalous condition through repeated experimentation. With other types of eggs, such as the green frog, leopard frog, and common toad, the percentage of production fell much lower.

The most conspicuous external feature of these tadpoles was a dwarfed appearance together with a relatively prominent and bulging trunk. The total length of the body was about one-fourth less than that of the controls, while the tail accentuated this shortening over the controls by as much as one-half. The head parts, though reduced in size, were well formed. This was especially true of the brain. Their swimming reactions were sluggish, resembling in this respect the above-mentioned tadpoles, but all movements were carried out normally.

A study of the histologic sections demonstrated features which so far as related to the cardiovascular and nervous systems were identical to those described in the previous paper. They require no added description here. At the junction of the trunk and tail, however, the anatomic arrangement merits special attention. At this level, extending at the most a distance of two or three segments, the neurocele was expanded. The major part of the dilatation affected chiefly its dorsal parietes, thereby producing an attenuated wall. The myotomes in the affected segments were normally formed, but lay somewhat farther laterally than is usually the case. This dorsal expansion of the neurocele had the effect of bringing its dorsal wall into close contact with the ectoderm, the ventral wall being in approximately its usual position relative to the notocord. A few mesenchymal cells occupied the narrow interval between the neural tube and the ectoderm. To all histologic appearances, the ectoderm was normal.

It was necessary to trace the several developmental steps in the production of this condition in order to ascertain by which of the two possible methods this anomalous condition had been brought about. The approximation of the neural tube to the ectoderm was not the resultant of a delayed separation of the neuroblasts of the neural groove from the latter. The early developmental stages of the neural ridges and groove, comprising the separation of the latter from the ectoderm, progressed in a perfectly orderly and normal fashion. This stage was followed in these defective embryos, as well as in the controls, by the interposition of a mesenchymal layer between the ectoderm and the neural tube. During those later stages, however, when the defective development of the heart was made manifest through dilated vessels, pericardium, and a static circulation, then the dilation of the neurocele at the segmental level mentioned became pronounced. This expansion affected the dorsal wall of the neural tube more markedly. Apparently, as a direct result of pressure thereby applied, the mesenchymal elements were forced out of the interval between the tube and the ectoderm. At the same time, the neural arch-halves were restricted laterally, as was true of the myotomes as well.

Extended experimentation with waves of varying length has demonstrated to the mind of the author the outstanding fact that if such a condition as specific cytologic susceptibility to x-ray activity exists, the greatest single factor to which that phenomenon may be referred is the relative mitotic activity of the various types of cells. That is to say, where a sufficient amount of x-ray energy of the usual wave-length is employed, it is possible to influence directly all types of cells of embryonic tissues. The single determining factors are, apparently, the quantity of energy utilized plus the rate of division of the cells. As development progresses, the varying degrees of absorption capacity are in direct proportion to the rapidity of mitotic succession in those cells. This accounts in part, at least, for the observed greater absorption capacity of young and growing neuroblasts, in contrast to the relative lack of that property in adult cells of the same type. That there has been in the experiments given a direct inhibitive action upon the neuroblasts is evidenced through both staining and morphologic features. But the physiochemical mechanism underlying the increased absorption by cells of a single localized area in the caudal extremity of the trunk does not appear through a study of the experimental data. The defects noted in the cardiovascular system constitute apparently an essential element as well in the production of this defect. In no specimen yet studied has the condition of syringomyelocele been produced without an additional manifest cardiovascular defect as well. The question naturally arising, as to whether the defect in both systems, the neural and the cardiovascular, represent a single phase of cytologic reaction to x-ray activity, still awaits demonstrative evidence.

Resumen por el autor, Otto F. Kampmeier

Un caso notable de asimetría de la región tiroidea asociado con la presencia de un quiste branquial.

En la región cervical anterior de un varón negro de veinte años, fallecido a consecuencia de tuberculosis miliar, ha encontrado el autor las siguientes anomalías: 1. Ausencia completa del lóbulo izquierdo e istmo de la tiroides, con una ausencia correspondiente de las arterias tiroideas superior e inferior; el lóbulo derecho presentaba tamaño normal. 2. Ausencia completa del músculo esterno-tiroideo derecho; el músculo correspondiente del lado izquierdo presentaba tamaño mayor que el normal. 3. Presencia de una sola paratiroides en la región tiroidea inmediata. El autor no halló otras paratiroides. 4. Presencia de una tiroides accesoria (de 1.5 cm. de diámetro) situada inmediatamente debajo del lóbulo derecho de la tiroides; la irrigación sanguínea y el drenaje de la glándula demuestran que no se trata de un lóbulo izquierdo de la tiroides desplazado. 5. Presencia de un quiste cervical o branquial rodeado de un epitelio (de 2.5 cm. de longitud por 1.2 cm. de anchura) el cual, situado al nivel del cartilago tiroides, estaba aplicado al lado izquierdo de la farínge extendiéndose hacia arriba hasta el asta mayor del hioides. El carácter de las asimetrías y anomalías mencionadas indica que se originaron en fases tempranas de la vida embrionaria, durante la época en que los derivados glandulares de la farínge y de la musculatura branquial y cervical comenzaron a formarse y diferenciarse. Del mismo modo no puede dudarse que en la producción de estas diversas anomalías estas estaban interrelacionadas de un modo definido durante su formación y que pueden referirse a un factor nocivo común.

A STRIKING CASE OF ASYMMETRY IN THE THYROID REGION ASSOCIATED WITH THE OCCUR- RENCE OF A BRANCHIAL CYST

OTTO F. KAMPMEIER

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ONE FIGURE

The following observations were made on a negro male, twenty years old, who died at the Cook County Hospital of miliary tuberculosis, and whose body was dissected during the past school year.

When the skin and subcutaneous tissues were being reflected from the region of the anterior triangle of the neck, nothing unusual was noted. Both the omohyoid and the sternohyoid muscles were broad and well developed and were situated in their normal position. But when these muscles were reflected a striking asymmetry of the underlying structures became apparent. The left lobe of the thyroid and the isthmus were entirely absent,¹ and on the right side there was no trace of a sternothyroid muscle, while the corresponding muscle on the left side was much larger than usual. Later, when a search was made for the parathyroids, only one was found in the immediate thyroid region. Still later a cyst was discovered behind the larynx to the left of the pharynx.

The right or existing lobe of the thyroid was of normal shape and size, and occupied the usual position as indicated in the figure (*thyr.*). Its surface showed distinct lobulations, it was firm, and in section showed no abnormal characteristics.

Situated at the inferior margin of the single thyroid lobe occurred a rounded mass of tissue (fig. 1, *thyr. acc.*) approxi-

¹ The writer thanks his students Messrs. Leitch, Lenzen, Goebel and Brough for calling his attention to the absence of the left lobe of the thyroid and for their carefulness in following his instructions in the subsequent dissection.



Fig. 1 The figure shows the skin and subcutaneous tissue removed from the region of the anterior triangle of the neck and the sternocleidomastoid, omohyoid, and sternohyoid muscles cut to expose the underlying structures, such as the trachea, thyroid, the carotids, jugulars, thyrocervical trunk and branches, etc. Approximately one-half natural size. *a. thy. sup.*, arteria thyroidea superior; *a. thy. inf.*, arteria thyroidea inferior; *v. thy. sup.*, vena thyroidea superior, this branch is absent on the left side; *v. thy. inf.*, vena thyroidea inferior (on account of its relatively high position, it might be called vena thyroidea media; the corresponding vein is absent on the right side); *v. thy. ima*, vena thyroidea ima; *m. st. thy.*, musculus sternothyroideus (absent on the right side); *thy.*, right thyroid lobe (left lobe absent); *thy. acc.*, accessory thyroid; *parathyr.*, cross-barred area indicates position of the only parathyroid found (beneath sternothyroid muscle); *cyst*, cross-barred oval area indicates position of the branchial cyst between larynx and pharynx.

mately the size of a marble, though somewhat flattened, which on microscopic examination of a section was found to be composed of thyroid follicles. At first it was believed that this small thyroid nodule represented a much reduced and displaced left thyroid lobe, but study of its vascular supply and drainage led one to a different conclusion. The right superior and inferior thyroid arteries (*a. thy. sup. et inf.*), which in their origin from the carotid and thyrocervical trunk, respectively, were normal, not only supplied the right thyroid lobe, but after ramifying through its substance sent small terminal branches to the inferior nodule. Moreover, the inferior thyroid vein (*v. thy. inf.*) of the left side drained only the thyroid lobe and not the nodule, while the thyroid ima vein (*v. thy. ima*) drained both. The right inferior vein was absent. These observations would seem to indicate that the small separate thyroid nodule, instead of being a reduced and displaced left lobe, represented an accessory thyroid, which condition occurs relatively frequently, as is well known, and which apparently results during the early development of the gland, when the proliferating cords of cells break up into the anlagen of the follicles and the ingrowing connective tissue divides the gland into more or less definite lobules. It is assumed, however, that in this case the formation of this accessory thyroid was related in some way to the causes which determined the asymmetrical development of the neighboring structures.

On the other side, no trace of a left superior thyroid artery was found, and what was believed to represent the vestige of a left inferior thyroid artery was so minute that it was impossible to follow it out to its termination. It was lost in the connective tissue lateral to the trachea and beneath the sternothyroid muscle (*m. stthyr.*).

The asymmetry of the sternothyroid muscle, normally paired, was very interesting. No vestige of this muscle was found on the right side; not even an aponeurotic band or strand was found stretching across the anterior or ventral face of the thyroid. On the left side, on the contrary, the sternothyroid muscle (*m. stthyr.*) was well developed and stretched between its normal

points of attachment on the manubrium sterni and the thyroid cartilage. It was thicker than usual and with the underlying connective tissue filled out the space normally occupied by the lobe of the thyroid. The thyrohyoid muscles were present on both sides.

In searching for the parathyroids, all the small nodules, six in number, in the immediate vicinity of the thyroid were removed and sectioned, after their positions had been plotted on a drawing. Only one of these structures, however, was found to be composed of parathyroid tissue; the remaining ones were small lymph nodes. This single parathyroid had the shape and size of a small pea, and was situated in the connective tissue immediately dorsolateral to the trachea at the level of the cricoid cartilage. Its position has been projected on the overlying sternothyroid in the figure (*parathyr.*). Other parathyroids, if present, must have occupied positions much farther removed from the thyroid than is normal.

The cyst mentioned above represented a flattened tough sac, rectangular in outline and approximately 2.5 cm. long, 1.2 cm. wide, and 0.6 cm. thick (dorsoventral diameter). It was located at the level of the thyroid cartilage against the left side of the pharynx, and its upper end extended as far (fig. 1, *cyst*) as the larger horn of the hyoid bone. The walls of the sac, although tough and resistant, were relatively thin, semitransparent or translucent, and showed a yellowish fluid content within its cavity. This sac was attached by two tough connective-tissue strands, an inferior one, passing downward a short distance and disappearing in the connective tissue between larynx and pharynx, and a superior one, 3 or 4 cm. long, joined to the fascia covering the pharyngeal musculature.

After the location, attachment, and topographical relations of the cyst had been noted, it was removed from the cadaver, opened, sectioned, and stained. Microscopic examination showed an epithelial lining, composed chiefly of a stratified epithelium of five or six layers of cuboidal or polygonal cells. In certain areas, however, the cells formed only a single or double layer. Outside this epithelium was a layer of fibrous connective tissue, not very wide, but rather compact and dense.

Considering the existence of several possibilities of origin, it was of course impossible to determine the exact embryological derivation of the cyst. It might represent the persistence of any one of the last two or three pharyngeal or branchial pouches of the embryo, or it might have developed from a persisting postbranchial body, a structure of problematical significance derived from the vestigial fifth branchial pouch and normally disappearing in the lateral lobe of the thyroid during the latter's development and expansion. It can hardly be assumed that the cyst may have developed from a part of the original thyroid anlage which had become isolated from the main mass during its migration from its initial site beneath the tongue to its definitive position, for the location of the cyst dorsal or posterior to the larynx would not support such an assumption.

The character of the asymmetries described above, especially the absence of any vestiges of the left thyroid lobe and the right sternothyroid muscle, would suggest that these abnormalities began early in embryonic life, at the time when the glandular derivatives of the pharynx and the branchial and cervical musculature began to form and differentiate. Which factor disturbed the normal developmental process in this region is impossible to say at the present time. But there seems to be no doubt that the production of these different abnormalities were definitely interrelated in their formation and can be referred to a common disturbing factor.

In perusing the literature on the abnormalities and malformations in the thyroid region, the author found only two or three cases in which the absence of one of the lateral lobes of the thyroid was reported, and only two cases in which the sternothyroid muscles were completely absent. In 1852, Handfield Jones classifies among other anomalies of the thyroid, exhibited in the museum of Guy's Hospital, London, a preparation in which only one lobe of the thyroid was developed, associated with a little glandular body situated below the middle of the thyroid cartilage. In 1862, Luschka, in his text-book of anatomy, reports the case of a complete absence of one lobe of the thyroid in a new-born. In 1883, Gow observed the absence of the left thyroid lobe in

an old woman. He finds the superior and inferior thyroid arteries of the corresponding side present, but very minute. He states that the right thyroid lobe was not enlarged, an observation also made by the present writer, who did not notice any compensatory hypertrophy of the remaining lobe. A condition apparently as rare as the absence of one of the thyroid lobes is the complete absence of the sternothyroid muscles, which has been noted by Otto (1816, 1824) and Chudzinski ('94). In these cases, the muscle was found absent on both sides.

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Resumen por los autores, Raymond L. Barney y Barry J. Anson

Abundancia estacional del pez destructor de mosquitos, *Gambusia affinis*, especialmente en relación con su fecundidad.

El estudio de cortes de los órganos reproductores de las hembras grávidas y no grávidas de *Gambusia affinis* ha demostrado la existencia de un tejido que envuelve exteriormente al ovario y la de un "conducto genital" funcional que conduce las larvas al seno urogenital después que han abandonado los folículos ováricos. Por medio de un exámen microscópico de los ovarios de unos 800 individuos capturados durante todos los meses del año los autores han obtenido pruebas que indican claramente que en Mound, Louisiana, todos los huevos de una *Gambusia* hembra de 3.3 cm. o mayor longitud, y tal vez los de las hembras de menor tamaño, que aparecen más tarde durante el mismo año en forma de larvas, son fecundados simultáneamente.

La producción de huevos en esta especie es un fenómeno cíclico, setando regulada la cuota anual de peces jóvenes por el tamaño y potencias metabólicas de la hembra, y no solamente por la temperatura del agua favorable para las actividades de la cría. Existe un periodo de descanso en el ovario que dura desde el nacimiento de las larvas a fines de Septiembre y en Octubre hasta la época de la fecundación, en la primavera siguiente. La relación del tamaño de la hembra y la temperatura del agua con la fecundidad es indicada por los autores. Se ha observado una correlación entre la rapidez estacional de la liberación de las larvas de *Gambusia*, la abundancia estacional y la "frecuencia de los jóvenes" de la especie en las condiciones naturales.

THE SEASONAL ABUNDANCE OF THE MOSQUITO-
DESTROYING TOP-MINNOW, *GAMBUSIA AFFINIS*,
ESPECIALLY IN RELATION TO FECUNDITY

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THREE FIGURES AND TWO PLATES (SIX FIGURES)

In previous papers the authors ^{1, 2} have shown the changing seasonal abundance of *Gambusia* and how varying frequency of potent males, oxygen content of water, plant associations, and other general environmental features affect the natural history of this viviparous top-minnow. The value of such knowledge in its relation to a practical mosquito-control campaign has been pointed out in this connection. As a supplement to these publications, the present paper is offered as a further consideration of the natural history of *Gambusia* with special reference to fecundity in its general bearing on seasonal abundance of this species in nature. This study corroborates and is correlated to some previous conclusions concerning changing seasonal abundance and gives certain added information concerning productiveness in this minnow.

The fish examined and discussed herewith were collected at Mound,* Louisiana, during the years 1916, 1917, 1918, and 1921. Examinations of the fish were made by the junior author and Mr. H. Walton Clark. Approximately 800 female *Gambusia*, collected from many points along bayous, borrow-pits, wood lakes, and pools, representing several different environ-

* The authors were employed as representatives of the U. S. Bureau of Fisheries in a cooperative study of fish control of mosquitos with representatives of the U. S. Bureau of Entomology. The observations were made from the latter's field laboratory at Mound. Special thanks are due Dr. W. V. King for suggestions and for the collection of certain of the *Gambusia* discussed in this paper. We are indebted to Dr. R. E. Coker for criticism of the manuscript and for suggestions.

mental associations discussed in previous papers, were examined. Counts were made of all embryos.

The anatomy of the female reproductive organs and the embryology of *Gambusia* are so unusual that a résumé of the facts bearing on these subjects seems warranted, in view of their relationship to further discussion in this paper. We take the liberty to quote in this connection the careful observations of Ryder⁷ (pp. 144 and 145):

The ovary is a simple, unpaired organ, the greater part of which lies on the right side of the body-cavity below the air-bladder, and serves to fill up the greater part of the inferior moiety of the former when developed to maturity with its follicles gravid with embryos. The ova, when full grown, are each enveloped in a sack or follicle supplied with blood from a median vascular trunk, which divides and subdivides as it traverses the ovary lengthwise in a manner similar to that of the stem to which grapes in the bunch are attached. . . .

Every fully-grown ovum, by means of the preceding arrangement has its own independent supply of blood from the arterial system of the mother, the ovarian arterial trunk being a branch of the median dorsal aorta. Each egg and egg-sac is thus supplied with materials for its growth and maturation, the latter eventually becoming specialized into a contrivance by which the lives of the developing embryos are maintained while undergoing development in their respective follicles. The young or unripe eggs which are found together in the same ovary with the developing foetuses are, as stated above, enveloped in a cellular and fibrous stroma, which serves not only to strengthen the vessels, but also afterwards enters into the structure of the walls of the ovarian sacs or follicles externally, as these grow in size . . . [See fig. 4.]

The ova after developing a little way are each enclosed in a follicle or ovisac, *membrana granulosa* of Von Baer, or *membrana cellulosa* of Coste. As the egg develops there seems to be a space gradually formed about it in the same way as described by Wyman in *Anableps*. This space is filled with fluid, and in this liquid, which increases in quantity somewhat as development proceeds, the embryo Cyprinodont is constantly bathed. [See fig. 5.]

There is no trace whatever in the egg follicles of Gambusia of an independent egg membrane, such as is present in the ovary of all known forms of osseous fishes which spawn directly into the water [See fig. 5.]

Kuntz⁵ (p. 184), in his study of the reproductive organs of *Gambusia*, claims, disagreeing with Ryder, that the ovary itself has an exterior investment which serves as an oviduct at its

posterior end connecting thus with the urinogenital sinus. Our observations indicate that there is an exterior investment of the ovary (fig. 6) and that there is also a functioning 'oviduct' (fig. 7) which conveys the embryos to the sinus when they have burst forth from the follicles. A better term than oviduct would be genital duct, since this organ conveys only larval *Gambusia* after they leave the follicular tissue.

The females chosen for examination in this study were of all sizes and were in all conditions of gravidity, carrying ova and embryos of all degrees of development. Each egg and embryo was examined under the microscope to eliminate any possible error as to the classification of the contents of the ovary as ova or early embryos. It is not difficult in this species to distinguish between a mature ovum and an egg fertilized within a few hours, especially after the fish has been preserved in spirits. The embryo appears, then, if in a blastodisc or blastoderm stage, respectively, as a white cap or a white streak of tissue. Not only are the embryos readily distinguishable by general appearance, but also, ordinarily, by their relative sizes. There is always a batch of minute ova embedded in the follicular tissue (fig. 4), but these are so small and opaque that they could never be mistaken for ova capable of fertilization. As the embryos of a large female, say, over 3.3 cm. in length, reach maturity and are liberated, there is a slight growth of the ova embedded in the follicular tissue; but in fishes of this and greater size, it is evident from our observations that these ova never attain full development, become mature, or are fertilized during the breeding season of that year.

There is, as has been said, especially in the large females, a considerable mass of embryos undergoing development, as Seale's⁸ (p. 181) data show. Fertilization, however, of all ova in females of appreciable size (3.3 cm. and larger), from our observations, occurs at approximately the same time. This conclusion is based on several facts.

In the first place, in females of appreciable size collected at any time from March 15th to October 1st, embryos are always found which are in very closely succeeding stages of development,

and never has there been found in the females examined a very or moderately advanced embryonic stage in the ovary simultaneously with a very early stage or with unfertilized but mature ova. Occasionally an abnormally developed embryo, embryos, or ova have been found. Abnormally developed ova and embryos have been observed in females examined in the proportions of one to twenty-six and one to seventy-five, respectively. The embryos were always malformed and the ova were always very few, the usual number being one or two. To cite typical cases: in a female measuring 3.85 cm. collected in September, 1917, one apparently abnormal ovum was found with thirty-three normal embryos; in a female 3.45 cm. in length taken in April, 1918, one abnormal embryo was found with eighty-two normal embryos, all in approximately the same stage of development. In seven of all females examined, ova measuring from 1 mm. to 1.4 mm. have been found with embryos in the same ovary. It is believed that this condition, being so different from that of the very large number of females examined, has resulted from an abnormal condition of the ovary or of the ova and does not signify a possible series of impregnations.

Again, if fertilization were not simultaneous for all the ova of a female's annual production, it would be expected that in some of the females examined during the year unfertilized but mature ova (a mature ovum measured 1.6 to 1.8 mm.) would be found at the same time as embryos. Such a finding would indicate the possible occurrence of waves of production of maturing ova and their subsequent impregnation and embryonic development, and would signify also that embryos in several well-separated stages of development would be found within the ovary of the same female. Such a condition, however, has never been found in the large number of females examined.

In fish examined which carried embryos, the ova which accompanied the pregnant condition were very minute, varying in diameter between the extremes 0.13 and 1 mm. (fig. 4), for the interval from March to February. These minute ova continue within these extreme measurements until about February 20th, when development begins, and in the largest fish, embryos in

early stages are found about March 15th. After fertilization the development of the embryos continues apparently simultaneously and in the same amount in each embryo until a rather late stage (fig. 8), from which point the final development is attained, in some earlier than in others. Those embryos which first obtain complete development are given birth and constitute the young of the first batch of the annual brood. The cause of the quickened development and of the resulting appearance of batches of the brood at intervals is problematical. This may, however, be due to the location of the embryos in the ovary. After the production of the first batch the embryos next to be liberated are given their final development and are liberated, and so on, until the entire annual brood is released.

Kuntz⁵ (p. 183), writing of the fecundity of *Gambusia* collected at Beaufort, North Carolina, states that—

In the same ovary may be found ova in various stages of development ranging from almost microscopic dimensions to a diameter of 1.8 millimeters attained at maturity. A considerable number of ova reach maturity at the same time. These being fertilized give rise to a brood of young. After the birth of this brood, another lot of ova reach maturity, and, being fertilized, give rise to a second brood. Thus, perhaps, all the ova required to produce the several broods which are born during a spring and summer may be present in the ovary at the beginning of the season.

The statement that mature ova are developed in waves of production and that liberation of a batch of young is followed by the fertilization of a batch of ova just matured, which will subsequently be the next batch of young liberated, does not find corroboration in our observations. However, we agree that all the ova which constitute the annual brood are in the ovary at the beginning of the season. Hildebrand⁴ (pp. 7 and 8), discussing the life-history of this minnow, points out that his experiments, carried on at Beaufort, showed that females, allowed to associate with males in the spring and then separated from them, continue to produce young fish throughout the season, during which at least five broods are liberated. He points out also that females allowed to associate with males through the summer and separated from them late in the fall

and then carried through the winter, did not produce young the following spring, and that the ova of these females were not fertilized. He concludes that the causes of these phenomena were the facts that spermatozoa could be carried by the female throughout the breeding season, but could not survive in the female during the winter period. His inference was based on an analogy between a description of the reproductive organs of certain other viviparous fishes of the same family, Poeciliidae, and his knowledge of those of *Gambusia*. More precisely, in the two viviparous forms which he mentions as related to *Gambusia* he says, quoting from Phillippi⁶—

Within the folds of the lining of the oviduct the sperms were found in great numbers, even after the birth of the young; . . . it is probable that the sperms are retained there throughout the breeding season and that the eggs are fertilized as soon as they are sufficiently matured.

Whether this supposed storage of spermatozoa occurs or not, the simultaneous fertilization of all the ova which will represent the year's subsequent production of young precludes the necessity for it. If there is storage, it would appear to be only another example of the excessive overproduction of the male sexual element and of the unnecessary frequency of copulation to insure maximum fertilization and survival of the species.

With further reference to the simultaneous fertilization of all the ova that may represent the year's subsequent production of young, the embryo counts have been divided into groups of collections occurring during periods of two months' time. They have also been averaged for each pair of lengths indicated, measurements being from the tip of the head to the base of the caudal fin (table 1). The number of embryos averaged and tabulated bi-monthly appears as shown in table 1.

The curves in figure 1, supplemented by the above discussion, indicate that female *Gambusia*, approximately 3.3 cm. and more in length and possibly those of smaller size, have all their ova, which result later in the season in young fish, fertilized simultaneously. Taking a concrete example based on averages computed from counts of the embryos of a considerable number of

TABLE 1
Seasonal *Gambusia embryo count, 1917, 1918, 1921*

	LENGTH														
	1.7-1.8	1.9-2.0	2.1-2.2	2.3-2.4	2.5-2.6	2.7-2.8	2.9-3.0	3.1-3.2	3.3-3.4	3.5-3.6	3.7-3.8	3.9-4.0	4.1-4.2	4.3-4.4	4.5-4.6
April and May { Fish Embryos				1 17		10 19.6	17 24.1	16 31.6	18 40.6	15 63.1	16 108.1	15 128.4	15 145.6	7 184.2	3 196.6
June and July { Fish Embryos			5 8.6	17 13.8	24 17.6	27 24.7	20 26.1	12 33.9	7 40.4	4 40.2	8 60.3	3 93.6	5 91.2	2 101	
August and September { Fish Embryos	1 9		9 9.1	9 13.5	21 15.4	10 20.5	11 26.8	4 23.2	6 41.6	4 40.2	8 40.5	4 49.5			
October to February { Fish Embryos	1 0	6 0	11 0	7 0	19 0	20 0	9 0	7 0	4 0	5 0	2 0	2 0			

females, the tabulated size 3.7 to 3.8 cm. is chosen as representative. From this it will be noted that in April and May the average number of embryos found in sixteen pregnant females was 108.1; for June and July, 60.3; for August and September, 40.5, and for October, November, December, January, and February, 0; in fact, in these five months there were no embryos found in females of any size. This, being true of all the females of appreciable size and being supplemented by observations previously discussed, indicates that all fertilization of the eggs occurred early in the breeding season, and immediately after copulation, with no needed storage of spermatozoa.

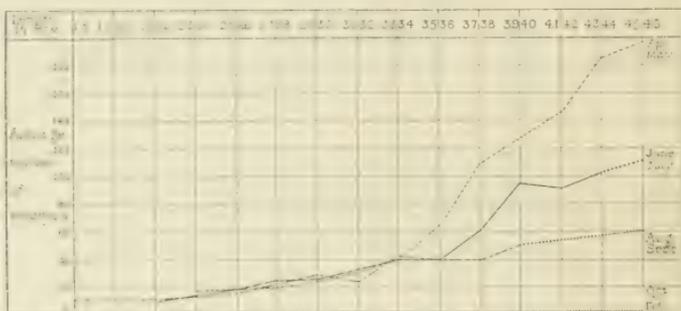


Fig. 1 Seasonal *Gambusia* embryo count. Estimated.

There is, therefore, in the case of *Gambusia* females of appreciable size a condition analogous to that found in many oviparous fishes, namely, that of having all the eggs capable of fertilization during the year fertilized simultaneously, and when these are laid or liberated no further production of young by the mother fish until the next year. This observation may or may not obtain for smaller females. Their very fast growth may possibly allow for two cycles of egg production within one year. It appears, then, from figure 1 that *Gambusia* females of appreciable size collected at Mound have but one annual cycle of egg production. This may hold true for smaller females, inasmuch as it has been noticed in this study that females of a size which would indicate that their birth occurred during the previous fall—that is, they

measured 2.4 cm. or less in length in March—neither carry embryos in March nor are their ova large enough to lead one to expect impregnation before at least six weeks. In no case were ova found in fishes of this size in March of greater diameter than 0.41 mm.

The relation of length of female to fecundity is best illustrated by the tabulated data for April and May (table 1). It will be noted that fecundity in this species increases markedly as the length of the female increases. From the average count of

TABLE 2
Gambusia frequency and varying influencing factors

	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Gambusia frequency, 1919.....		13.7	9.9	12.1	22.5	39.2	52.2	122.9	69.6	38.5	20.0	23.1
Young frequency, 1919		0	0	4.0	5.0	10.2	10.2	20.6	15.8	2.6	1.3	3.2
Male frequency, 1919.		34.0	27.3	23.5	16.1	21.9	13.4	10.6	29.2	20.2	23.0	26.7
Average air temperature, 1915 to 1919, inclusive.....	46.4	52.1	59.5	63.9	71.0	78.4	80.3	79.8	73.4	65.7	55.4	48.5
Per cent of pregnant females 1917-1919..	0	0	35	?	40	58	73	81	69	0	0	0
Per cent of total liberation of young....					31		62		100			

embryos based on April and May observations, the fecundity of females of 2.3 to 2.4 cm. length is doubled when the fish is about 3.2 cm. long; the fecundity is about quadrupled when the length 3.5 to 3.6 cm. is reached. With each 2 millimeters' growth thereafter the fecundity is greatly increased. The largest embryo count of this study was 226 for a female 4.3 cm. in length in May. The smallest female observed to be carrying embryos was 1.7 cm. in length and was carrying nine embryos in August.

From March through August there is a regularly increasing percentage of females that are carrying embryos. It appears that the proportionate number of females and males is such that

there is, at the beginning of the breeding season, a number of females that have not been impregnated. As the season progresses, however, the percentage of impregnated females increases, one copulation being sufficient for fertilization of all mature ova of that female. There is, then, a steady accumulation of pregnant fish during the spring and summer.

The percentage of unfertilized females in the spring and early summer is increased by those relatively few females born during the past late fall, which just reach sexual maturity and are

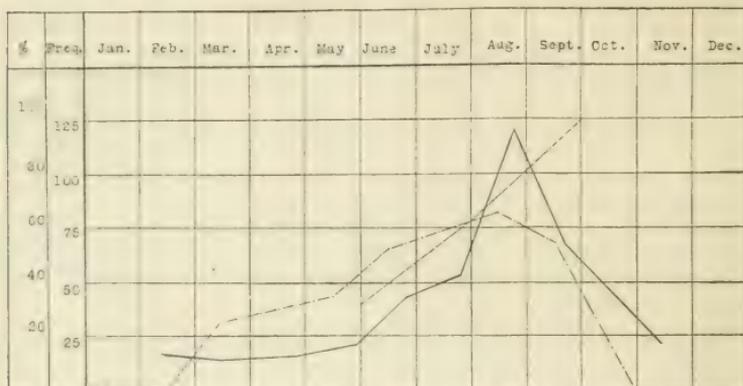


Fig. 2 Seasonal frequency in relation to fecundity in *Gambusia*. ——— *Gambusia* frequency; ----- Per cent of liberation of young; - - - - - Per cent of pregnant fish.

impregnated for the first time during the late spring. Figure 2 indicates the close relationship between the monthly percentage of pregnant fish and the changing '*Gambusia* frequency' curve of our recent paper¹ (p. 60). The highest frequency of *Gambusia* in our monthly observations at Mound occurred in late August. The highest percentage of pregnant females occurred at the same time. It appears, therefore, that the high frequency of *Gambusia* in midsummer results directly from the increasing percentage of pregnant females up to and including this time.

Realizing that the increased frequency of *Gambusia* at any time depends for the most part upon the prolificness of the larger females, especially since the production of young by the younger and smaller fish probably equals or merely balances the destruction of the species by natural causes, a correlation is noted between the bi-monthly record of liberated young (fig. 2), for a given size (the length 3.7 to 3.8 cm. being used as representative) and the monthly increase in *Gambusia* frequency. At the height of *Gambusia* frequency about 80 per cent of the year's offspring has been liberated.

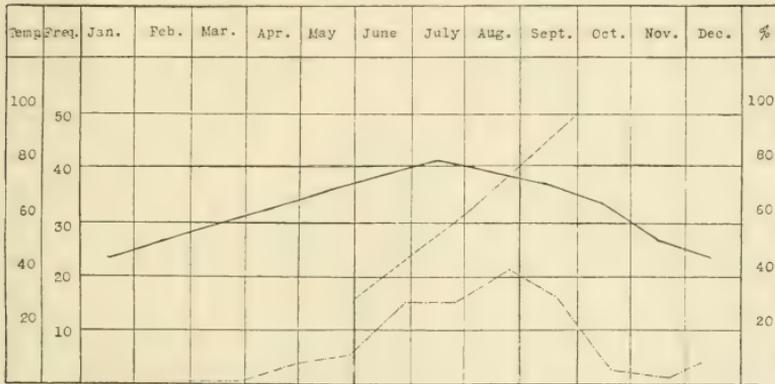


Fig. 3 Temperature in relation to fecundity in *Gambusia*. ———— Temperature of air; ----- Per cent of liberation of young; - . - . - Young frequency.

The seasonal liberation of young is dependent on the temperature of the water in which the fish live, other conditions being equal. The temperature record of table 2 is the monthly average mean temperature of the air as observed at Mound from 1915 to 1919, inclusive. There is no record available of water temperatures of all bodies of water concerned herewith. However, the close relationship of water temperature with that of the air for this vicinity is noted in a recent publication³ (p. 251, fig. 3). It appears that liberation of young is rapid through August, the last warm month of the summer, but that thereafter there is very little further liberation. In fact, as has been said, by the

1st of September at least 80 per cent of all births have been accomplished. Collections made after the 1st of October failed to reveal a single embryo. That there may be a few young born after October 1st is indicated, however, in the 'young frequency' of 1919 for the Mound vicinity¹ (p. 58, table 2).

After the last embryo has been liberated by the mother fish in the fall, even though the temperature is favorable for further development of eggs or for copulation and fertilization, development and impregnation of ova do not occur. Having collected young of the year as early as the middle of April, it is believed that the fertilization which initiated the development of these young must have occurred at least three or four weeks before this time. It is probable, in view of Seale's observations⁸ (p. 181) on the period of gestation, that fertilization among these fish occurred even much earlier than four weeks previous to the birth of the first young. The average mean temperature of the air at Mound for the month of March is 59.5°F., the same for October and November is, respectively, 65.7° and 55.4°F. It would appear probable that copulation and fertilization which may occur in March at the above-mentioned temperature could well continue at least until the 1st of November. The fact remains, however, that this does not occur. Metabolism of the fish cannot be markedly lowered by decreasing temperature or by lack of food before the 1st of November, since both of these factors remain favorable to this date. These facts suggest that temperature is of influence only in increasing the metabolism of the fish and is not significant in lengthening the breeding period. It appears, therefore, that egg production in *Gambusia* is a cyclical phenomenon, the annual quota of young being a certain number governed by the size and metabolic potentialities of the female for that season. The period from the first birth in April to the last in September or October is virtually a protracted period of gestation and parturition. The time intervening between the last liberation of one year and the fertilization of the next spring appears, then, whether temperature and feeding conditions are favorable or unfavorable for the maturing of eggs or the fertilization thereof, as a period of rest and recuperation for another

year's long period of pregnancy. This is readily suggested, also, by observations on the condition of ovaries during the period from October to January. During these months no ovum larger than 0.41 mm. in diameter was found in a total of sixty-three individuals examined. The modal size of these ova was 0.2 mm., and in each fish examined the ovary was very elongate and much reduced in size (fig. 9).

Referring again to the seasonal birth of offspring in this species (fig. 3), a positive correlation is noted between it and the 'young' of the previously mentioned paper on changing frequency in this species' (p. 67). 'Young' production, as observed at Mound, increased from late March to August, at which time the figures representing percentage of young liberated are at their highest. As might be expected, the percentage of pregnant fish is greatest in August.

SUMMARY

1. In addition to a tissue investing the ovary of *Gambusia affinis*, there is also a particular organ, the genital duct, which conveys the larval *Gambusia* from the ovary to the external sinus.

2. Evidence is cited indicating that the ova of all females of appreciably large size—3.3 cm. and more in length—in the vicinity of Mound have all their ova which appear later the same year as liberated young fertilized simultaneously.

3. Increasing size of females and coincident increasing fecundity of *Gambusia affinis* as observed at Mound, Louisiana, have been tabulated and discussed.

4. The relation of temperature of the water to the liberation of young and fecundity of *Gambusia* at Mound is indicated. It appears that 80 per cent of the annual production of young occurs before any considerable decline in the temperature of the water is noted.

5. Egg production in *Gambusia* is a cyclical phenomenon, the annual quota of young being a certain number governed by the size and metabolic potentialities of the female for that season, and not alone by temperature.

6. The seasonal rate of liberation of young *Gambusia* at Mound is positively correlated with seasonal abundance and with 'young frequency' of the species.

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PLATES

Photomicrographs (figs. 4, 5, 6, 7, 8, and 9) were taken by Mr. J. B. Southall, of the U. S. Biological Station, Fairport, Iowa.

PLATE 1

EXPLANATION OF FIGURES

4 Dorsoventral sagittal section of ovary containing developing embryos. Fish collected July 3, 1916; size, 3.5 cm. to base of caudal fin. Section shows developing embryos resting on yolks which float in follicular sacs. Near center of cut there appears a number of immature ova embedded in the follicular tissue. Stain, eosin. $\times 12$. Thickness, 50μ .

5 Dorsoventral median sagittal section of urinogenital system of a female *Gambusia* (same as no. 4), showing urinary canal, genital duct, and mouth of intestine. Lower embryo clearly shown floating in the follicular sac. Embryonic tail has been cross-sectioned. Stain, eosin. $\times 12$. Thickness, 50μ .

6 Dorsoventral section near median line (same as nos. 4 and 5), showing investment of the ovary and a broadened portion of the genital duct. Stain, eosin. $\times 7$. Thickness, 50μ .

7 Longitudinal section of non-gravid female collected March 10, 1921; size, 3.5 cm. to base of caudal fin. Section above the level of the colon showing folds of the genital duct, unfertilized ova embedded in the follicular tissue, and the urinary duct posterior to the genital duct. Stain, iron-alum haematoxylin. $\times 9$. Thickness, 50μ .

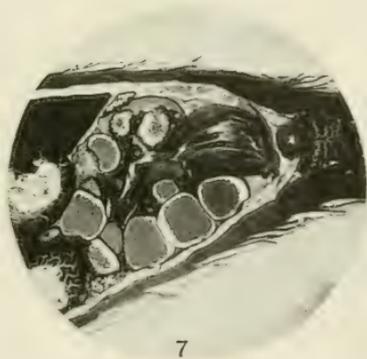
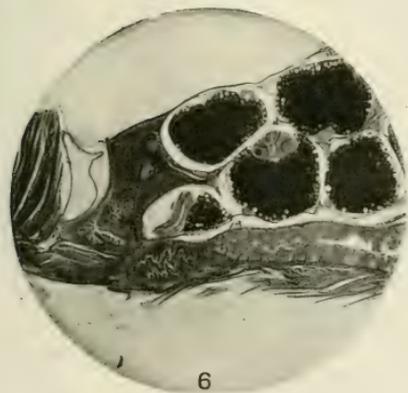
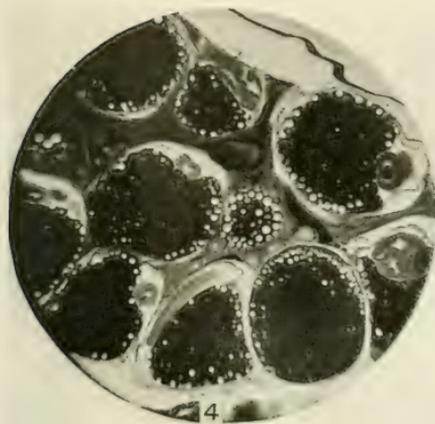
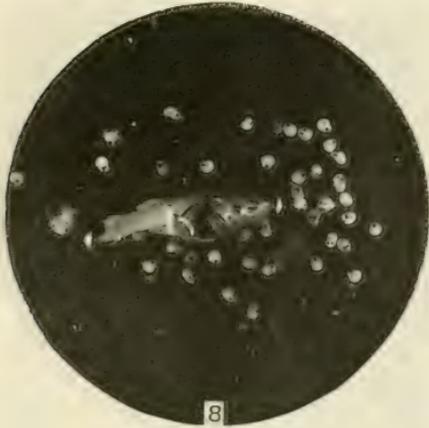


PLATE 2

EXPLANATION OF FIGURES

8 View of female, 2.75 cm. to base of caudal fin, collected September 13, 1918, with forty-one embryos dissected from the ovarian follicles. These embryos are all in approximately the same stage of development and represent probably the last two liberations of the annual brood. Approximately natural size.

9 Dorsoventral sagittal section of a female, 2.9 cm. to base of caudal fin, collected February 14, 1921, showing ovary in whose follicular tissue are contained numerous immature ova. Note the reduced size of the ovary and the very small ova. Stain, eosin. $\times 7$. Thickness, 50 μ .



9

Resumen por los autores, Carl Caskey Speidel y Roy M. Hoover

El método de la gelatina-glicerinada montada en un marco para la preparación y montaje de extensos cortes o de material patológico.

Mediante este método se pueden montar y conservar permanentemente extensas secciones de tamaño adecuado, tales como cortes transversales de un niño recién nacido, siendo de fácil manejo para demostraciones y estudio. Cada sección se coloca en una marco especial de madera, en el cual se ha fijado una placa de vidrio, que sirve de fondo del recipiente. El marco se llena con una mezcla de gelatina glicerinada y ácido fénico, que se vierte caliente, solocando el corte y cubriendo con una segunda placa de vidrio, que se fija por medio de listones de madera, completando de este modo el marco.

La gelatina se solidifica al enfriarse quedando incluída la sección en una masa de gelatina dura y transparente cubierta por las dos placas de vidrio y limitada lateralmente por el marco de madera. Los bordes de este se sumerjen en parafina fundida para evitar el acceso de aire. El material patológico puede también prepararse de este modo, así como secciones de cuerpos humanos jóvenes o de otros vertebrados de tamaño conveniente. Las principales ventajas del método son la eliminación de líquidos conservadores y evitar la posible mutilación o desintegración del material al manejarle.

THE GLYCERIN-GELATIN PICTURE-FRAME METHOD OF PREPARING AND MOUNTING GROSS SECTIONS OR PATHOLOGICAL MATERIAL

CARL CASKEY SPEIDEL AND ROY M. HOOVER

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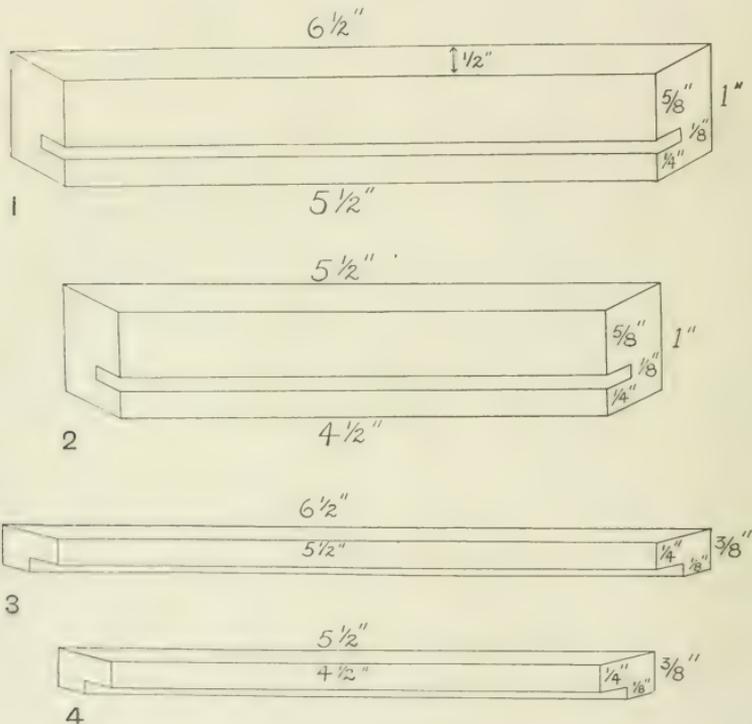
FOUR FIGURES AND ONE PLATE (FIGURES 5 AND 6)

Cross sections of the human body, such as are generally used for study in a course in topographic anatomy, do not last very long in good condition. Handling by the students inevitably results in the disarrangement and mutilation of the structures of the sections. The following method was used in an attempt to prepare sections in such a way that they could be kept permanently in first-class condition, but still be available at all times for study.

For this purpose an infant (which had died at birth) was used. Warm carmine-gelatin solution was injected into the blood-vessels by way of the femoral artery. This injection mass congealed as the body was cooled. The body was then placed in a jar of full-strength formalin after first injecting some of the formalin into the abdominal, cranial, and thoracic cavities to insure penetration. After a couple of days the body was sufficiently hard for sectioning. Sections were made by placing the body in a miter-box and cutting with a heavy, long, sharp knife. The cross-sections were made about $\frac{1}{2}$ inch in thickness.

The sections were now to be mounted in a warm glycerin-gelatin solution which would harden when cooled. For permanent preservation the mount must be made air-tight, in order to prevent drying of the gelatin. Special frames were made for this purpose. Two long strips of pine wood were obtained. Strip A was 1 inch wide, $\frac{1}{2}$ inch thick, and with a groove $\frac{1}{8}$ inch wide and $\frac{1}{4}$ inch deep, placed as in the diagrams (figs. 1 and 2).

Four pieces with mitered ends were cut from this strip; two were $6\frac{1}{2}$ inches in length (fig. 1) and two were $5\frac{1}{2}$ inches in length (fig. 2). These were nailed together to form a rectangular frame with a glass plate fitted into the groove, the whole resembling somewhat an ordinary picture frame.



Figures 1 to 4

In like manner four pieces of corresponding lengths were cut from strip B, which was $\frac{3}{8}$ inch wide, $\frac{1}{2}$ inch thick, and with a groove $\frac{1}{8}$ inch wide and $\frac{1}{4}$ inch deep, placed as in the diagrams (figs. 3 and 4). Together with another glass plate they formed the rest of the frame and were to be added after the cross-section and the glycerin-gelatin solution had been introduced.

Glycerin-gelatin solution was made up according to the following formula:

Water.....	42 cc.
Gelatin.....	6 grams
Glycerin.....	50 cc.
Carbolic-acid crystals.....	2 grams

The gelatin was soaked in water for one-half hour, then dissolved with gentle heat. Five cc. of white of egg were added and the mixture heated (the egg albumen gradually precipitating, carried down all fine particles of dust, etc., so that the gelatin was left perfectly clear). The gelatin was then filtered through moist cotton and the glycerin and carbolic acid added. The mixture was then warmed for fifteen minutes and stirred until homogeneous. (The gelatin should not be heated above 75°C., or it may not harden again at ordinary temperatures.)

A layer of this glycerin-gelatin solution was poured upon the first glass plate, then a section which had been previously dipped in the solution was laid upon it, then more of the glycerin-gelatin added until the frame was completely filled. The second glass plate was next fitted over the frame, all air being carefully excluded, and the four pieces from strip B nailed in their proper positions over the plate, thus completing the frame. The glycerin-gelatin mass hardened as the section was cooled. In this manner the section was completely embedded in hard clear gelatin mass, with a glass plate above and below and a wooden frame around the edges. It remained only to dip the edges of the frame in melted paraffin to make the whole mount absolutely air-tight and consequently permanent.

The photographs of plate 1 (figs. 5 and 6) show two views of a finished mount. Figure 5 represents an oblique side view. Figure 6 is a front view and shows a cross-section of an infant at about the level of the third lumbar vertebra. The photograph, however, gives little idea of the color contrasts that are displayed by the actual section.

This method could not be so easily applied to cross-sections of adult bodies, because of the size of the sections. The larger the glass plates are, the more easily they may be cracked or broken.

We believe, however, that for the smaller bodies of half-grown persons the method would be a very satisfactory one. Sections of adult human brains also make good material for mounting in this way. Good serial cross-sections of other vertebrate animals of suitable size (such as rabbits, cats, dogs, etc.), as well as of human foetuses, could also be obtained by this method. And finally, many gross pathological specimens could be mounted in this way in frames of suitable size and be conveniently available for purposes of demonstration or study.

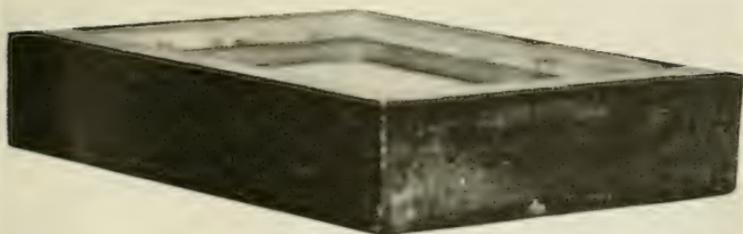
The elimination of preserving fluid and the elimination of the possibility of mutilation or distortion of the sections or specimens by handling constitute the chief advantages of this glycerin-gelatin picture-frame method.

PLATE 1

EXPLANATION OF FIGURES

5 An oblique side view of a finished mount, showing the structure of the frame. Photograph.

6 A front view of a finished mount, showing a cross-section through an infant at about the level of the third lumbar vertebra. Photograph.



5



6

Resumen por el autor, Ludwig A. Emge

Notas sobre el estudio de las mitocondrias en el amnios humano.

Los gránulos mitocondriales aparecen en las células del amnios humano en todos los estados de la gestación. Bajo el punto de vista morfológico son semejantes a las mitocondrias que se encuentran en otros órganos en todos sus rasgos, con excepción de su afinidad por las materias tintóreas, que es menor que la de las mitocondrias en otros tejidos. Su disposición está influida por la forma de la célula, pero es constante para grupos definidos de ciertos tipos de células. El periodo de gestación no influye sobre el tipo, forma, tamaño, disposición o número de las mitocondrias, con la excepción de los amnios muy jóvenes que el autor ha estudiado.

Translation by José F. Nonidez
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NOTES ON THE STUDY OF MITOCHONDRIA IN THE HUMAN AMNION

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TWO FIGURES

The search for mitochondria in the human amnion was undertaken for purely anatomical reasons, in order to prove or disprove their presence and to establish such morphological variations which may facilitate future investigations. It is well known that certain types of these granules exist in other units of the gestation product. Van Cauwenberghe found mitochondria in the cells of the chorion and the placental villi during the first half of gestation. Later, Kervily demonstrated them also in the same structures during the second half of pregnancy, laying Van Cauwenberghe's failure to find them during the second half to insufficient chromization of the tissues studied. Kervily concluded that morphologically these granular structures were alike at the various periods of gestation. While studying the amnion I have had occasion to verify Kervily's findings, and can confirm his contention that mitochondria of quite similar appearance are present in the cells of the chorion and the placental villi at all stages of gestation. On the other hand, our own technical observations do not confirm Kervily's assumption that Van Cauwenberghe's failure was due to imperfect chromization, since we saw these granular structures equally often if chromization was materially shortened.

TECHNIQUE

The study of the material includes twenty human amnions fully mature and five younger amnions of $1\frac{1}{2}$, 4, 5, 7, and 8 months of age, respectively. Of the first group five were obtained

at caesarean sections before the membranes had ruptured. The location of the tissue removed from all membranes was carefully noted, and during the early part of the study various locations were studied in each amniotic sac. This latter procedure has proved to be of greatest importance, since there is a definite difference in the appearance of groups of cells within one amniotic sac. The greatest uniformity is present in the area covering approximately the inner two thirds of the placenta. For a comparative study of human amnions this is the only area which will give sufficiently uniform results to permit conclusions, and this rule for selecting tissues should be observed for all amnions more than five months old.

Since Kervily emphasized prolonged chromization of the tissues in order to arrive at the best results, small pieces of amnion obtained directly at birth were fixed in Regaud's neutral formaldehyde-bichromate mixture, which was changed daily for six days. The tissues were then chromicized in 3 per cent potassium bichromate for one week. (Osmic acid could not be obtained at the time of this study.) All tissues were collected in body-warm fluids and the containers kept in an ice-box for twenty-four hours. They were then transferred to a dark locker and kept at room temperature for the remainder of the period. The exclusion of light, and especially sunlight, is of importance in obtaining even penetrations with fixing agents containing potassium bichromate. I arrived at this conclusion by purely physicochemical reasoning. As a rule, such fixing fluids remain clear in the ice-box, and to some degree when light is excluded at room temperature, while, when they are exposed to bright light or sunlight or when they are kept in the incubator at 37°C., the fluids show a heavy precipitate. This means that certain rays and certain temperatures rapidly reduce the potassium bichromate. When this occurs, one finds a heavy water-insoluble layer around the tissue which prevents or materially retards the further process of fixing and mordanting. Since I have observed this occurrence, I have strictly adhered to fixing under the exclusion of direct light, and I am convinced that I have obtained tissues more uniformly penetrated by these fixing agents.

In the course of this study the period of fixing and chromicizing was gradually reduced to twenty-four and forty-eight hours, respectively, and it was found that this time is entirely sufficient to obtain satisfactory results. I, therefore, cannot join Kervily in his contention that Van Cauwenberghe's failure was due to an insufficient period of chromization. In this laboratory we practice secondary chromization as advocated by Bensley if we feel that poor staining results are due to imperfect mordanting. This method of chromicizing the mounted section for a few minutes before staining has proved very valuable in our hands. Nevertheless, staining difficulties will be encountered continually when it comes to staining the amniotic cell. This holds true for both vital and other stains. The only explanation that can be offered is that the plasma of this cell has a low chemical or physical affinity for the conventional fixing agents as well as for the mitochondrial stains. The prohibitively high price of other metals used in fixing fine cell structures has kept me from looking further into this question. The staining of the amniotic cell for mitochondria, either in the vital or fixed state, is a difficult problem indeed. I have not been able to discover the reason, but it remains a fact that this cell as well as all of its finer details have a poor affinity for dyes.

After trying out various methods, it was found that modified acid fuchsin mixtures, such as described by Altmann and by Bensley, would give the best results to demonstrate mitochondria, although even these 'best results' were not very satisfactory. It is of no use to describe the modifications, as they vary with each individual piece of tissue and depend upon the ingenuity of the morphologist in knowing how to adapt staining methods.

Vital dyeing was found to be unusually difficult. The vital dyes diffuse the cell plasma so quickly that it is often impossible to make out individual structures. To obtain a clear cell picture is a matter of luck in spite of the greatest care in not allowing the tissues to dry and in attempting to keep them at an even temperature. The saline solutions which were used as solvents for the dyes were varied in their composition, but this did not materially affect the end results. There was no better success when amni-

otic fluid was used as a solvent. After finding that all dyes related to neutral red and methylene blue were useless on account of their tendency to stain granules of various nature, Janus green B in 1:4000 Ringer's solution was used as a criterion. In tissues exposed to this dye, basal striations, apparently made up of mitochondria, could be seen occasionally in certain types of cells; in other types, masses of what seemed to be mitochondrial substance were observed. But the really finer types of granules could not be made out on account of the difficulty stated above. The only definite statement that one can make in regard to vitally stained amniotic tissue is that mitochondrial granules are present at all stages of gestation described here. A comparative study between fresh and fixed tissue beyond the question of existence of these granules in the amnion proved to be fruitless.

A STUDY OF THE FIXED MATERIAL

Among twenty-five amnions studied there were twenty mature membranes, five obtained at caesarean sections, before rupture, and one each from a pregnancy terminated therapeutically at 1½, 4, 5, 7, and 8 months. The outstanding factor is that there are several types of mitochondria in the cells of all these membranes, but that there is no essential difference in the morphological appearance of the individual types of granules in corresponding types of cells at the various periods quoted. A slight variation is found only on comparing the granules of the earliest to the fully mature membranes. While the relative number of mitochondria in the given cells of these membranes is apparently proportionally the same, one does notice a greater uniformity in the size and the shape of these granules as a whole in the earliest three amnions described here. The explanation for this may be the greater uniformity of amniotic cells as such at these periods. In the second half of gestation groups of cells within one amnion vary a good deal in appearance, which finding is most marked 'at term.' In the mature membrane there are found, side by side, groups of high and low columnar and cuboidal cells, each group varying somewhat in the detail architecture of its individual cells.

Nevertheless, after allowing for these slight variations, one can establish a mitochondrial criterion which is applicable to practically all groups of cell types in the individual membranes.

In the first half of pregnancy the globular and cocci-shaped mitochondrial granules are the most common forms present. In the second half rod-like and filamentous shapes become more conspicuous, and in the fully mature membrane masses suggesting mitochondrial substance are common. While the types of mitochondria are fairly uniform under similar conditions, the distribution of these granules varies within individual cells of the same type. Only in the earliest amnion studied a uniform distribution of the mitochondrial granules was found throughout the individual cell. In the cells of all other amnions the distribution depends definitely upon the shape of the cell and its location in regard to certain points of pressure.

I call attention to the fact that certain locations in the amnion do not represent the true general type of the amniotic cells and its internal architecture. For instance, each membrane has certain areas in which cell life is either interrupted or actually at its end, if one may judge from morphological appearances. The extreme of this trophic disturbance is best seen in an area varying from 8 to 10 or more cm. in diameter, which apparently represents the point of greatest amniotic pressure. Here the cell structures have lost most of their staining affinity. If one may use the expression, it is only the shell of the cell that accepts some dye. Usually the cells of this area are flattened out. Their nuclei are indistinct or necrotic. Mitochondrial structures are commonly absent or exceedingly scarce, and in the latter case will not hold the dye. My first impression was that this area was always located over the internal os of the cervix uteri, since it was found here in all of the unruptured membranes obtained at caesarean sections. That this assumption is not correct was demonstrated later by a comparative study, when it was found that this area may be situated at higher levels and that it corresponds to the site of the spontaneous rupture of the membrane. Usually there are several of these areas demonstrable which vary in degree of trophic changes. It is true that in multiparous

women such an area is commonly present over the internal os of the cervix, although this may not be the future point of rupture, and therefore at least two atrophic areas may be encountered in one membrane. In primiparous women the location of these areas may be found anywhere. The presence of these areas is constant in all membranes and suggests that the spontaneous rupture occurs at the point of greatest disintegration of cells. Since the outline of this area or areas is irregular, it was found advisable to secure tissues a good distance away from the point of rupture. The best and uniform tissues were obtained from that part of the amnion which covers the inner half of the placenta. I have dwelt so lengthily on this factor because if this rule is not observed future studies must end in conflicting observations.

The distribution of mitochondrial structures in the membranes of the second half of gestation is relatively uniform in corresponding types of cells, as pointed out above. Here one often notices a distinct bipolar distribution of mitochondria with a rather marked difference in the appearance of the granules at each pole. The largest amount of mitochondria is seen in the basal third of the cell regardless of what shape the cell may be. The closer the granular structures approach the actual base of the cell, the oftener they are seen to assume a formation of basal striation. This is most pronounced in the high columnar and piriform cells. This striation is made up of short parallel thin rods of equal length. The individual rods which bulge slightly at their midpoints rarely traverse more than one-sixth of the entire length of the cell and most commonly occupy about one-tenth of the long diameter of the cell. When the cell approaches the lower types of columnar shape, the striation begins to move away from the base. At the same time the staining intensity decreases and some of its definition is lost until, when the cell becomes very low, it is lost entirely. Mitochondrial structures are then replaced by a conglomerate mass that has all the staining characteristics, vital and fixed, of mitochondrial substance. Janus green B is taken up more readily by this substance than it is by defined mitochondrial structures. When exposed to any

of the mitochondrial solvents the mass disappears, which leads me to believe that it is either a massing of mitochondria prior to dissolution or that it is a direct and very early mitochondrial derivative. This mass also has a marked affinity for fat dyes



Fig. 1 Photomicrograph with oil-immersion lens. Represents the rod-shaped mitochondria of the columnar cell types. Note the regularity with which they are arranged in basal striation. The cupolas of the cells are connected by fine plasma bands. Few spherical mitochondria can be seen in several cell cupolas.

Fig. 2 Photomicrograph with oil-immersion lens. Represents the low columnar and cuboidal cell type in which the mitochondrial substance has lost its definition and forms a conglomerate mass away from the base of the cell.

about in the same proportion as mitochondria have when they undergo dissolution. Such masses are rarely seen in the high types of cells and are also uncommon in the very lowest types. Most commonly they are seen in the cuboidal and low columnar types.

Towards the mid level of the cell mitochondrial structures become very scarce. The only exception to this is an occasional very slender cell standing distinctly alone and supported only by very delicate plasma strands bridging to adjacent cells. In this type of cell long, slender filamentous mitochondria traverse the entire length of the cell. Individual filaments gracefully embrace the nucleus, leaving a very narrow clear zone in its direct vicinity, and enter the cell cupola to form a densely interlaced labyrinth. In all other cells there is, as a rule, a fairly wide nuclear zone which is free from mitochondria. This zone varies in size and position with that of the nucleus. It is widest if the long axis of the nucleus is parallel to that of the cell and narrowest if the two axes intersect at right angles. A fair appreciation of this can be gained only if the cell is seen in its true profile. Most often this zone occupies the upper end of the middle third of the cell, but it may actually move into the upper third and even enter the cell cupola.

While in the basal third of the cell rod-shape types of mitochondria are the outstanding structures with the globular and filamentous forms in the minority, the reverse is true of the upper third of the cell. In fact, here rods are so rare that they are negligible and filamentous forms occur only in the certain slender cells described. Most commonly, one finds the spherical, small, cocci-like types, but in contradistinction to mitochondria in the basal third these globular and cocci-like structures are ill-defined. They are few in number, vary considerably in size, usually are located in the joints of the cellular network, which renders them still more indistinct, and tends to form a very thin conglomerate layer directly under the membrane of the cupola. Occasionally, one sees strands of these conglomerates enter the intercellular bridges.

Aberrant forms of mitochondrial structures may be found in any type of cell, but their number appeared to be so small that it seemed to be insignificant to describe them. No attempt has been made at this time to make a very close comparison of the earlier amnions in regard to mitochondria, because the material is too scanty to obtain definite comparative pictures. The

description of these, therefore, has been confined to the most general statements made in the first part of the report.

CONCLUSIONS

In conclusion it is stated:

That mitochondrial granules occur in the cells of the human amnion at all stages of gestation;

That, with the exception of their staining affinity, which is less than in other tissues, they morphologically resemble in every respect mitochondria found elsewhere;

That their arrangement is influenced by the shape of the cell, but that this arrangement is quite constant for definite groups of certain types of cells;

That, with the exception of the very earliest amnion studied, the period of gestation has no influence on the type, shape, size, arrangement, or number of mitochondria seen.

I wish to express my appreciation to Miss Ella Wing, who looked after the cutting and mounting of the material studied.

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Resumen por los autores, J. A. Myers y Frank Myers

Estudios sobre la glándula mamaria.

VII. La distribución de la grasa subcutánea y su relación con las glándulas mamarias en vías de desarrollo del macho y la hembra de la rata albina desde el nacimiento hasta las diez semanas de edad.

El depósito de la grasa subcutánea es más marcado en la vecindad de los conductos lactieíferos—formados y en vías de desarrollo. Desde el nacimiento hasta las diez semanas de edad, la grasa subcutánea se distribuye del mismo modo tanto en los machos como en las hembras. Los autores creen que el depósito sirve como protección contra el frío y como reserva alimenticia. La ausencia de una capa adiposa protectora distribuida uniformemente en la rata puede estar relacionada con el desarrollo precoz del pelo y la costumbre de los animales de vivir en espacios cerrados. En las ratas imperfectamente nutridas desde el nacimiento, el depósito de grasa subcutánea decrece y el desarrollo de los conductos lactieíferos se retarda.

Translation by José F. Nonidez
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STUDIES ON THE MAMMARY GLAND

VII. THE DISTRIBUTION OF THE SUBCUTANEOUS FAT AND ITS RELATION TO THE DEVELOPING MAMMARY GLANDS IN MALE AND FEMALE ALBINO RATS FROM BIRTH TO TEN WEEKS OF AGE

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THREE FIGURES

While fixing the glands for fat stains in the study of witches' milk (Myers, '19), it was noticed that when the entire skin of an albino rat was removed, stretched out on cork, and placed in Flemming's fluid for a short time, the subcutaneous fat was beautifully differentiated from the surrounding tissues. There seemed to be a constant relation between the deposit of subcutaneous fat and the development of the mammary glands. Hence the present work was undertaken to determine this relation.

MATERIAL AND TECHNIQUE

Representative stages from birth to ten weeks were selected from apparently healthy albino rats of normal body weight. For each stage a male and a female from the same litter and of approximately the same body weight were studied and compared. The rats were killed by chloroform and an incision was made extending from the dorsal part of the root of the tail along the spines of the vertebrae and over the skull to the tip of the nose. The skin was then reflected, special precautions being used to remove all the subcutaneous fat with the skin. The skin was then stretched out on a piece of cork with the inner surface up, after which it was placed either in Flemming's fluid or 1 per cent osmic-acid solution. The preparations were left here until the subcutaneous fat had become dark brown. This usually required

thirty minutes to one hour. They were then washed in running water for an hour or so and carried through the various grades of alcohol. It was noticed that in skins left much longer than one hour the epidermis became somewhat darkened so that the subcutaneous fat was no longer so sharply outlined. After the material was hardened in the alcohol, drawings were made of the entire skin with the subcutaneous fat represented in black, the position of each of the twelve nipples being indicated on the drawing. These preparations did not show the ramifications of the milk ducts, hence it became necessary to remove the osmic-acid stain and make a study of the relation of the ducts to the fat. First, the connective-tissue layer of the skin was removed according to the method employed in an earlier work (Myers, '16). This thin layer, which contained the fat and milk ducts, was placed in a weak solution of hydrogen peroxide until the osmic-acid stain had entirely disappeared. It was then stained in very much diluted Delafield's hematoxylin and cleared according to the method described by Lane-Clayton and Starling ('06). These cleared preparations were then projected onto the original drawings in such a manner that the nipples coincided with the points indicating their position on the original drawing. This made it possible to outline the general distribution of each gland, thus showing the relation of the milk ducts to the subcutaneous fat.

Since osmic acid stains only the unsaturated neutral fat, triolein, the findings with osmic acid were verified by staining gross specimens with sudan III or scarlet red.

As soon as the skin was removed from the body of the animal, the entire body was placed in 1 per cent osmic acid to make sure that all subcutaneous fat had been removed. In most cases very little or no fat was left on the body wall of the animal.

In another series of animals the subcutaneous tissue was left on the body wall when the skin was removed. These bodies were then placed in osmic acid, scarlet red or sudan III, and the fat studied in its normal position. The results were similar to those with the other methods.

Microscopic sections were made through certain regions in order to verify the observations made upon the preparations stained in toto. Some of these sections were made from tissues fixed in Flemming's fluid. Frozen sections made from each stage studied were stained in sudan III or scarlet red and counterstained in hematoxylin.

OBSERVATIONS

The stains employed failed to reveal any subcutaneous fat in gross preparations from animals killed immediately after birth. In specimens taken *six to ten hours after birth*, however, a considerable amount of fat is seen deposited in the subcutaneous tissue. This fat is not uniformly distributed. In gross specimens this subcutaneous fat does not appear in solid masses at this stage, but appears somewhat granular. In a general way it may be said that the fat is deposited in the area supplied by the larger arteries and their branches. Therefore, one finds fat deposited more abundantly in the inguinal region around the inferior epigastric artery and its rami and in the thoracic and upper abdominal regions around the superior epigastric and mammary and intercostal arteries. In the region of the ventral midline, and for some distance to the side of this line, no fat appears. In the midabdominal region where the superior and inferior epigastric branches become very small, fat is not deposited at this stage. The relation of this early fat deposit to the blood-vessels is very constant. Along the larger branches the fat is quite plentiful, but it gradually disappears as one passes along the rami of these branches.

The nipple of the abdominal mammary gland lies medial to the fat being deposited in the inguinal and lower abdominal regions. Its primary duct, however, passes caudolaterally and divides into two or three branches which enter the region of the depositing fat. Here these branches subdivide, and all the terminal ramifications of the abdominal gland are found in the fat deposit. The first and second inguinal glands lie medial to the posterior continuation of the region in which fat is being deposited. The terminal branches of their ducts, however, ramify in this fat.

In the cervical region is an area in which fat is being deposited that extends somewhat cephalad and laterad from the root of the neck. There is some indication of small strands of fat connecting this area with the thoracic area which extends caudad very nearly to the costal margin and dorsad where it closely approaches the midline of the back. The nipple of the first thoracic gland lies medial to the axillary region, and fat is being deposited between this nipple and the axilla. The primary milk duct from this gland passes cephalad where it enters, and its rami terminate around the fat being deposited. The second and third thoracic nipples lie medial to the fat being laid down in the thoracic region. The branches of these glands also terminate around this fat.

Thirty hours after birth the subcutaneous fat is present in greater quantities and extends over larger areas than in the preceding stage (fig. 1). In the inguinal and lower abdominal region the deposit of fat is so sharply outlined that it may be designated as the inguinal fat pad. This pad of fat on each side extends from a position anterior to the posterior portion of the crest of the ilium toward the midventral line. Before reaching this line, however, it turns caudad, passes over the pubis, and extends nearly to the anal region. That pad of fat in the region of the iliac crest has become so thick, and yet is so unevenly distributed, that in gross stained preparations transmitted light penetrates only in certain areas. However, as one passes toward the pubic region and caudad to this, one observes the fat in smaller amounts so that it has somewhat of a granular appearance. Extending from the pubic portion of this fat pad may be seen thin narrow strands of fat extending cephalomedially to a point near the umbilicus. At this stage the inguinal fat pads of the opposite sides are not connected. The inguinal fat pad does not extend medially as far as the abdominal nipple. In case of the first and second inguinal nipples, however, this pad of fat has extended very nearly to their lateral margin.

Cephalad to the inguinal fat pad is a considerable area of subcutaneous tissue which in the previous stage contains no visible fat. However, at this stage one sees an occasional very

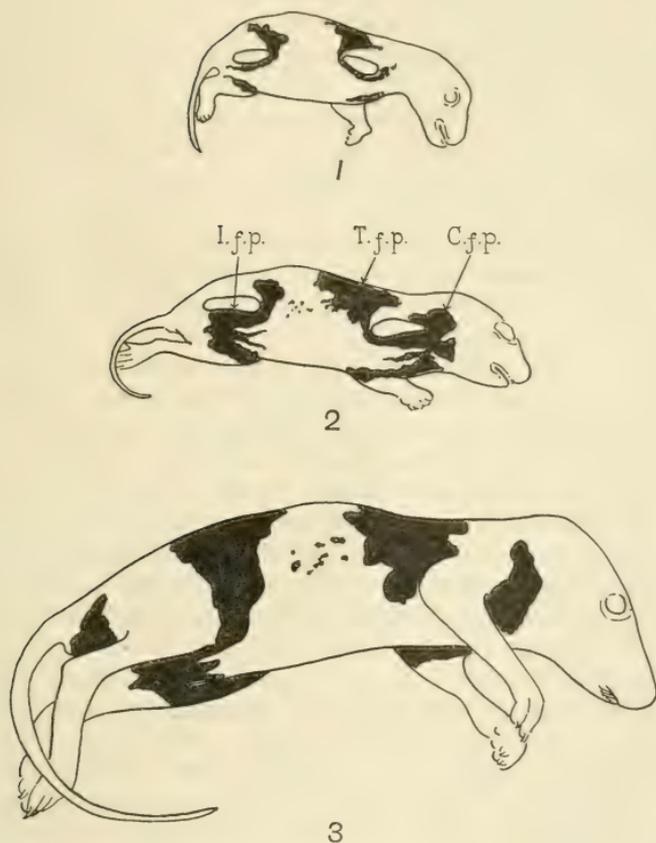


Fig. 1 shows the distribution of subcutaneous fat in an albino rat thirty hours after birth.

Fig. 2 shows the distribution of subcutaneous fat in an albino rat five days after birth. *C.f.p.*, cervical fat pad; *T.f.p.*, thoracic fat pad; *I.f.p.*, inguinal fat pad.

Fig. 3 shows the distribution of subcutaneous fat in an albino rat three weeks after birth.

small mass of fat in close relation to a blood vessel. As the thoracic region is approached, the subcutaneous fat is seen deposited in considerable amounts. In fact, one may now describe a thoracic fat pad. However, its boundaries are not so sharp as those of the inguinal fat pad. This is particularly true of the caudal boundary. The caudal boundary of the thoracic fat pad extends from a position very near the dorsal midline ventrally along and slightly below the costal margin. The medial boundary lies a few millimeters from the ventral midline, while the cephalic boundary passes just caudad to the axilla. This fat pad had increased in size so that its medial margin approaches very closely the lateral margin of the base of the nipples of the second and third thoracic glands.

The mass of fat being deposited in the cervical region in the previous stage has become quite definite in outline, and from this time will be designated the cervical fat pad. It extends from the anterior portion of the axilla into the root of the neck, where it turns laterodorsad and extends to the laterodorsal side of the neck. Caudally this pad of fat connects with the thoracic pad by means of narrow strands. The nipple of the first thoracic gland lies at the caudal end of the cervical fat pad, and its base is partly surrounded by this fat. The ducts of this gland ramify in the cervical fat pad.

Fifty-two hours after birth the fat pads are more distinct than in previous stages. They have not only thickened considerably, but extend over somewhat larger areas. The inguinal fat pads are slightly connected at their posterior ends. The cervical fat pads are also connected by small strands that extend across the midline. The inguinal fat pad has extended toward the midline until the nipples of the first and second inguinal glands are nearly surrounded by fat. The nipple of the abdominal gland still lies slightly medial to this pad. The strand of fat which in the previous stage extended from the pubic region to the region of the umbilicus has become more prominent. In the preceding stage attention was called to the fact that in the subcutaneous tissue lying between inguinal and thoracic fat pads an occasional small isolated mass of fat was observed. In the present stage such masses of fat are more numerous.

In the case of the nipples of the second and third thoracic glands somewhat isolated masses of fat may now be seen lying medial to their bases. The nipple of the first thoracic gland is so completely surrounded by fat that one is unable to see the position of the nipple in stained preparations. The cervical fat pads are slightly connected across the midline.

Five days after birth (fig. 2) the view of the bases of all the nipples except the abdominal, when examined from the inner surface of the skins, is completely obscured by the dense deposit of subcutaneous fat. In other words, the fat pads have extended toward the midline sufficiently to completely surround and cover up the bases of the nipples. In case of the nipple of the abdominal gland, the fat very closely approaches the lateral side of its base. The isolated masses of subcutaneous fat between the inguinal and thoracic pads have increased considerably in size. However, there is no direct fatty connection between these two pads at this stage. The cervical pads are now connected by good-sized masses of fat.

At the *end of the first week* the fat pads do not differ from the previous stage, except that they have become much thickened and slightly more extensive. The masses of fat between the thoracic and inguinal pads have increased in size so that in some specimens a few of them have fused to form a narrow connection between these two pads.

From the beginning of the *second week to the tenth week* (fig. 3) the subcutaneous fat pads increase in size with the general growth of the animal. They also vary somewhat in size depending upon the animal's health and food supply. During this period strands of fat may be observed extending across the midline connecting the cervical fat pads of opposite sides. Similar strands appear in the perineal region connecting the caudal portions of the right and left inguinal fat pads.

The milk ducts about the time of puberty undergo marked proliferation (Myers, '16) and it is interesting to note that their ramifications follow for the most part the same course of distribution as the subcutaneous fat followed at an earlier stage. Thus at this time milk ducts of the opposite sides cross the mid-

line in both the cervical and perineal regions and ramify in the fat already deposited in these regions. Other ducts send out branches which ramify in the processes of fat extending from the inguinal fat pads to the region of the umbilicus. Although strands of fat connect the thoracic and inguinal fat pads on each side, no ducts have been observed to ramify in these strands; so there is a definite interval existing between the ducts of the third thoracic gland and the abdominal gland on each side.

The ducts of the mammary glands of male rats after the fifth week do not grow as fast as those of females (Myers, '17), but no corresponding difference between the series was observed in the deposit of subcutaneous fat. The males at all ages show deposits similar to those of the females.

DISCUSSION

We believe neutral fat is deposited in the subcutaneous tissue for at least two purposes: first, as a protection against cold; second, as a reserve supply of food and energy. According to Berg ('11), much fat is deposited in the subcutaneous tissue of the new-born child. The question arises as to why so little or no subcutaneous fat is present in the rat at birth. In the first place, this animal is born in a very immature stage. Being born, however, without any special mechanism against cold, neutral fat begins to deposit beneath the skin very soon.

Again one might ask why this subcutaneous fat is not distributed more uniformly over the body. To be of much value as a protection against cold, one would expect to find a fairly uniform layer of subcutaneous fat as in some other animals. The answer to this may be found in the fact that the rat develops a fairly heavy coat of hair quite early in life, hence there is not much need for a further protective layer of fat. Moreover, the habitat of the animal may have some influence on the need of a protective layer of fat. For example, the rat usually inhabits fairly warm places, such as houses and cellars. It further protects itself by burrowing.

Attention has been called to the fact that the first subcutaneous fat is deposited in the neighborhood of the branches of the milk

ducts. Next, fat is laid down in greatest quantities in regions which will later be occupied by milk ducts. For example, the fat pads, at first small, become extended over a slightly larger area than the ducts will occupy during pregnancy and lactation. It was pointed out (Myers, '16) that the first cervical glands slightly before or at the age of puberty send branches across the midline so as to establish at least a gross continuity between the glandular tissue on one side with that of the opposite side. The same was true in case of the branches of the second inguinal glands. In both of these positions we see fat extending across the midline during the first week. In no other position at any time before puberty does fat extend across the midline.

Why should subcutaneous fat be deposited in definite pads in regions where the milk ducts later ramify? Is it because fat is a good medium for the ducts to proliferate in, or does it have some special rôle to play in the later functional stages of the milk ducts during pregnancy and lactation?

While studying the effects of inanition on the developing mammary glands (Myers, '19), it was observed that the subcutaneous fat that appears very early in the neighborhood of the milk ducts soon becomes greatly decreased after the amount of food is reduced to a minimum. In those animals that were underfed from birth very little subcutaneous fat was deposited. Along with this scanty deposit of subcutaneous fat occurs a partial failure of development on the part of the milk ducts.

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Resumen por el autor, John M. Tufts

Algunas observaciones sobre la estructura de las fibras de Purkinje.

Al revisar la literatura sobre la estructura del sistema senoventricular, uno no puede menos de notar dos características: Primera, la escasez de la literatura, y segunda, las grandes diferencias de opinión con respecto do la estructura de dicho haz. Comenzando con la bien conocida descripción de las fibras por Purkinje en 1845 y continuando el exámen de la literatura hasta las últimas contribuciones de Van der Stricht y Todd (1920) puede notarse que no hay dos investigadores que hayan llegado exactamente a la misma conclusión relativa a la estructura del sistema. En la presente breve revisión de la literatura solamente se indican las principales diferencias notadas en las contribuciones.

Translation by José F. Nonidez
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SOME OBSERVATIONS UPON STRUCTURE OF THE PURKINJE FIBERS

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THREE FIGURES

In reviewing the literature upon the structure of the sino-ventricular system one is impressed by two characteristics: first, the paucity of the literature and, second, the wide differences of opinion regarding the structure of the bundle.

Beginning with the well-known description of the fibers by Purkinje in 1845 and continuing through the literature till the last contribution by Van der Stricht and Todd ('20), no two observers have reached exactly the same conclusion as to the structure of the system. In the present short review of the literature only the chief points of difference in the contributions will be noted.

At the end of his memoir, Purkinje described a network of gray, gelatinous fibers. These fibers were said to be applied to the internal wall of the heart and to pass through slits in the wall. Microscopically they were said to be composed of polygonal cells, shaped thus by reciprocal pressure. They were said to contain one or two nuclei. Between these cells a network of striated fibers not independent of the cells was said to exist. Speculating upon the physiology and histology of this apparatus, Purkinje finally concluded that it is muscular in nature.

Ranvier ('83) stated that the fibers of Purkinje are little translucent cords, anastomosing with each other and thus forming a reticulum, the meshes of which are very variable. In sheep, where there is no fat under the endocardium, the fibers are said to show less by their translucence, but appeared on the internal surface of the heart in light relief. On a slide they showed as

polygonal cells arranged in the form of a pavement epithelium. Following the branching of the bundle to the finest fibers, Ranvier found them to be composed of a single row of cells. The larger branches were composed of cells lying side by side and above each other. A transverse and longitudinal striation was found at the periphery of the cell and a granular mass of protoplasm which lay in the center had one or usually two nuclei.

The striation seemed to be continuous from cell to cell, but the latter could be separated by maceration with 40 per cent picric acid. The separate cells showed granular striations all around and pigment granulations in the protoplasmic center. The fibers were continuous with the cardiac musculature and the end fibers showed transition stages. Ranvier found them to have a connective-tissue sheath corresponding to a sarcolemma and regarded them as embryonic muscle fibers which had been arrested in development.

Schweigger-Seidel ('70), in his description of the heart as reported in translation in the Proceedings of the New Sydenham Society devoted to Stricker's Human and Comparative Histology, stated that

1. The transversely striated muscle of the endocardium occurs in two forms. Purkinje fibers or a wide-meshed network of muscular bundles, broader and shorter than the second group, or
2. Gray, gelatinous fibers described by Purkinje in 1845 as located under the endocardium. These Schweigger-Seidel regarded as a motor apparatus composed partly of an embryonic form of muscular tissue. According to him, these fibers unite to form meshworks and are composed of more or less prismatic segments 0.05 to 0.1 mm. in diameter with a cortical layer of transversely striated musculature and a hyaline axillary material containing one or two clear nuclei. Schweigger-Seidel stated that some observers regarded the transversely striated mass as an intermediate substance containing isolated cells, while others regard each unit as a muscle cell in which, as in a certain stage of development, only the peripheral layers have undergone conversion into contractile substance.

3. These fibers pass into ordinary muscle bands, and in some animals their place can be taken by muscle bundles.

4. A division of the stronger fibers does not occur, and they are surrounded by a connective-tissue sheath. When injected they form a wide-meshed network.

Lehnert ('68) expressed the view that the cells are masses of protoplasm contained within bundles of striations which are therefore *extra-* or *inter-* and not *intracellular*.

Retzer ('06-'08) stated that the Purkinje fibers differ markedly in various mammals. They are most like ordinary heart muscle in man and differ widely in animals such as the sheep. The structure of the bundle was also found to differ in various portions of the same heart.

The text-book descriptions of the bundle contribute little to the determination of its structure, being chiefly taken from the above and other descriptions. Poirier and Charpey ('12) followed Ranvier's views, but Testut ('11) regarded these fibers as cells of cardiac musculature arrested in development. Schäfer ('12) concurred in this, but in Piersol ('13) it merely is stated that "Their significance has only been recently recognized. They are now regarded as the terminal part of an elaborate system of special muscle fibers whose probable function is the coordinative connection of the auricular and ventricular musculature that otherwise are distinct and unconnected."

De Witt ('07) stated that the sinoventricular connecting system of the human heart is composed of the 'Knoten' and the main bundle which remains undivided for a distance of 11 mm. Division occurs at the point of union of the posterior and median flaps of the aortic valve and at the upper extremity of the ventricular muscular septum so that the right and left limbs are sub-endocardial. These limbs then branch and extend into various parts of the heart. Here they become Purkinje fibers, and as such are not composed of cells, but are syncytial in structure. De Witt's reason for arriving at this conclusion was that—

1. The fibrils and fibril bundles pass uninterruptedly through the fiber in different directions, forming a more complicated fibrillar network throughout the fiber than could be accounted

for by any other hypothesis than that the fiber is the unit of the system, and that—

2. White or elastic connective tissue was not found penetrating the fiber or dividing it except that a few peripheral strands seemed to do so, which appearances easily can be explained by the fact that the former follow the irregular contour of the fiber strand. Neither did blood vessels or nerves seem to penetrate the fiber bundles.

De Witt also saw the clefts mentioned by Tawara, but believed them to be due to shrinkage of sarcoplasm during fixation. The cell-like forms or sarcoplasmic territories were found to be very variable in form and size and were separated simply by strands of cross-striated fibrils.

Van der Stricht and Todd ('20) described the Purkinje fibers as containing three types of cells: 1) Polyhedral cells embryonic in type, with the long axis somewhat exceeding the rest; 2) cells of the adult type much longer than the foregoing, many of which are particularly bulky, and, 3) Purkinje cells transitional in type showing stages of transition between the Purkinje elements and myocardial cells.

They further stated that the Purkinje cells of adult type and those of transitional type are placed end to end to form Purkinje fibers, extending parallel to the axis of the atrioventricular bundle and being bound together by short oblique branches. The short cells of embryonic type were said to form a network, the fibers or trabeculae of which are much shorter and the meshes narrower. All these elements were said to be delimited laterally by the interstitial connective tissue which fills the spaces of the network. The ends of the cells were said to be separated by intercalated discs across which the myofibrils pass continuously from one cell to another. These transverse discs presented the same structure and the same significance as those of cardiac fibers, but were said not to traverse the entire thickness of the Purkinje cell. They were said to be formed by thick segments of myofibrils and by a special clear substance. Where these discs were seen from the surface it could be observed that the myofibrils were condensed into a system of thick bands which

anastomosed to form a network in the spaces of which a clear fluid existed. Van der Stricht and Todd could not say whether this liquid represents a part of the sarcoplasm or an intercellular substance, but they felt that the solution of this problem would permit us to decide upon the syncytial or cellular nature of heart muscle fibers.

Regarding the "Delimitation of Purkinje cells of adult type," Van der Stricht and Todd said: "We have already seen that cells of embryonic type are generally isolated laterally by an endomysial membrane and are placed end to end although separated from each other by an intercalated disc. This disc is always traversed by myofibrils continuous from one cell into the other. Precisely the same arrangement is found in the case of the elements of the adult type. . . . On account of this precise delimitation, the Purkinje fibers formed by the juxtaposition of cells resemble fibers of the myocardium in which, however, the vascular interstitial connective tissue is much more abundant."

Under the heading, "The sarcolemma of the Purkinje cells," they wrote: "We shall content ourselves with stating that the Purkinje cells possess no trace of a true cellular membrane formed by their cytoplasm but that they are circumscribed laterally by a membrane-like connective system or endomysium which often figures as a false festooned sarcolemma."

King ('15) thought that the system was composed of separate cells which could be isolated, and it was in order to follow up this idea that the following investigation was made. Professor Meyer suggested that if isolated cells could be teased out in their entirety as King held, and stained, thus revealing a complete and independent cellular structure, this would quite clearly demonstrate the cell as the true unit of structure of the system. In acting upon this suggestion, comparatively fresh tissue from hearts of bovines and sheep killed the same day or at most within the preceding twenty-four hours were used. If the material was over twenty-four hours old, difficulty was encountered in making preparations, as the separate cells could not be teased apart without destroying their integrity. The hearts were

opened after the method described by Knowler ('08) and the system well displayed. The Purkinje fibers are easily seen as described, especially on the papillary muscles and the false tendons.

Preparations were made also from human hearts. These resembled closely those made from the beef and sheep hearts, but since the material could not be obtained soon enough after death these preparations are not described, for they revealed nothing new.

The endocardium was raised with a pair of fine forceps and dissected from over a part of a Purkinje fiber. The latter was then dissected away from the cardiac musculature with a pair of fine needles and quickly removed to a watch-glass containing either glycerin or normal salt solution. In some cases tap-water was used.

The fiber was now teased out under a lens, mounted on a slide, and stained by drawing stain under the cover-glass. The stains giving the best results were dilute solutions of methylene blue, methyl violet and Delafield's haematoxylin and Van Gieson.

Maceration of the tissue in various reagents such as 0.5 to 2 per cent nitric acid, dilute sodium hydroxide and picric acid also was made use of. The nitric acid gave the best results. It made the teasing out of the separate cells easier and interfered the least with the structure and staining reactions of the cells. In using nitric acid, portions of the fiber were macerated for twenty-four hours, washed with distilled water, teased out, and stained as above.

Another method used was to stain portions of the fiber in Delafield's haematoxylin, pass them through graded alcohols up to 35 per cent strength and leave them in the latter for forty-eight hours. After this they were macerated in water for several hours and teased, giving good preparations. A series of over one hundred teased preparations of various parts of the bundle and its ramifications was made, and the following examples taken from my notes will serve to illustrate the results obtained.

Preparation I was taken from the left ventricle of a sheep's heart where a false tendon left the septal wall. Polygonal cells could be isolated by teasing in tap-water. Several single cells

were separated from the groups of cells detached by this treatment. On staining the preparation with dilute methylene blue what appeared to be cell membranes could be detected. Centrally located nuclei could now be seen in most cases and several double nuclei were observed. Nucleoli were present, being elongated in form.

Preparation II. Sheep's heart. A specimen was taken from the upper part of the right ventricle near the first branching of the bundle. It was teased out and stained with dilute methylene blue. Large polygonal cells were again observed in groups and as separate cells.

Preparation III. Sheep's heart. A specimen was taken from the anterior papillary network. Separate cells were demonstrable. Some of this tissue was macerated and treated with gentian violet. The cells stained very quickly and deeply.

Preparation IV. Beef heart. A specimen was taken from the anterior papillary muscle near the base. It was teased out in salt solution and stained with methylene blue. Separate cells were again demonstrable. These varied in size and shape from almost round to four- and five-sided cells. Two nuclei are present as in the fibers of the sheep heart. Large elongated nucleoli are present and stain darker than the nuclei. The nuclei take the stain more deeply than the granular protoplasm. They are centrally located in almost all of the cells. There appeared to be a definite cell membrane. When pressure was applied to the cover-glass the cells stuck together at first, but then separated, as if some intercellular cement were present. When separated the edges of the cells were smooth and showed no ragged outline or teeth suggesting the ends of torn fibrils as might at first thought seem likely.

Preparation V. Beef heart. A specimen was taken from the muscle of the left ventricle. The fresh teased preparation was stained in 2 per cent osmic acid. Separate cells were observed with distinct boundaries. The cytoplasm stained and was granular with a clear area at the periphery. No nuclei stained.

Preparation VI. Beef heart. A fresh teased preparation was made, stained in Delafield's haematoxylin, and left in 35 per

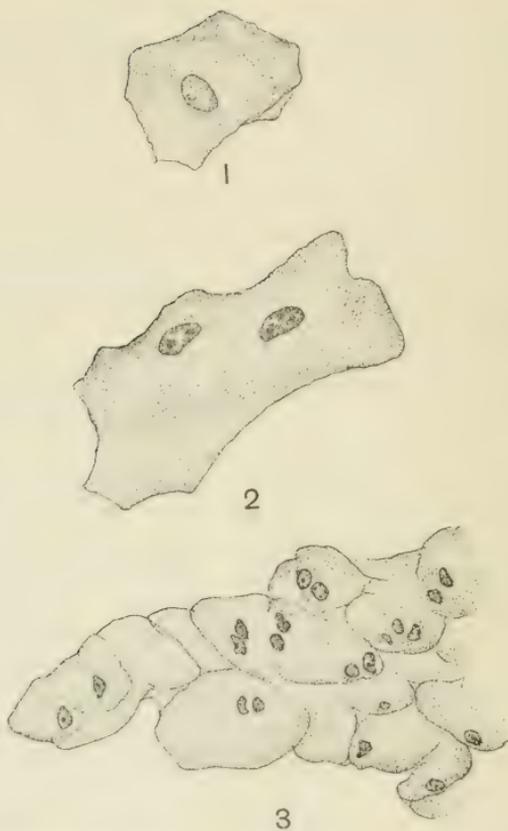


Fig. 1 Drawing of a single cell teased out from a section of Purkinje fiber taken from the papillary muscle in the left ventricle of a beef heart, stained with gentian violet. It shows the polygonal shape, granular cytoplasm with granulations more numerous at the periphery of the cell, nucleus and definite cell boundary.

Fig. 2 Drawing of a single cell from the same preparation as figure 1. Two nuclei are seen with nucleoli. The cell has a definite cell boundary.

Fig. 3 Drawing of a group of cells from a portion of a Purkinje fiber taken from the wall of the left ventricle of a beef heart. Partially teased out in tap-water and stained with gentian violet. It shows several cells with two nuclei and the granular cytoplasm especially marked at the periphery of the cells.

cent alcohol for forty-eight hours. It was then macerated in water and showed typical cells with what appeared to be a membrane and nuclei and nucleoli in each cell. Many cells possessed two nuclei and had a granular appearance.

In teasing out separate cells it was noted that the portion of the fibers used for teasing were in the form of circular rods composed of these cells. If a dissecting needle was rolled over this rod so as to break off a portion of the fiber, fibrils could be observed projecting from the torn ends of the rod. These fibrils showed cross striation upon staining with Van Gieson.

In an effort to ascertain the relationship of these fibrils to the cells several series of sections were made of pieces of the Purkinje fibers taken from the papillary muscle of beef heart. This material was fixed in Zenker's solution and imbedded in paraffin in the usual manner. Sections were cut both parallel to and at right angles to the long axis of the fiber from 2 to 4 μ in thickness and stained with Delafield's haematoxylin, counterstained with eosin or Van Gieson.

From these sections nothing definite as to the structure of the Purkinje fibers could be obtained other than what has been described by previous observers. Definite cells could not be made out in the sections. With a Van Gieson counterstain areas could be seen, with double nuclei containing an elongated nucleolus, in an area of clear cytoplasm and surrounded by an area containing striations seemingly continuous with those in the next cell. However, in the mounted sections no distinct division could be made out between the cells nor could the fibrils be demonstrated as being extracellular.

The preparations here described were duplicated many, many times and can easily be duplicated by anyone following the simple technique given. From the evidence so obtained it seems to me that the conclusion is justified that the cell is the unit of structure of the system. This cell, although large, varies greatly in size, however, in the same heart. The isolated cells appear to have a definite cell membrane surrounding them and vary in shape from almost spherical to rectangular or polygonal in form, suggesting the original statement of Purkinje that they are shaped

by reciprocal pressure. The cytoplasm is granular, the granules staining black with osmic acid. As observed by several investigators, many of the cells have two nuclei which are located centrally. These nuclei contain large, elongated nucleoli staining with ordinary nuclear stains, such as Delafield's haematoxylin.

From my observations on these cells in fresh teased preparations, I do not believe them to be embryonic muscle cells arrested in development.

In conclusion, the writer wishes to express his thanks to Doctor Meyer for suggesting the subject of this communication and for his assistance in carrying it to conclusion.

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Resumen por el autor, Wayne J. Atwell

La morfogénesis de la hipófisis en los anfibios caudados.

El autor ha estudiado la hipófisis en *Amblystoma*, *Spelerpes*, *Necturus* y *Amphiuma*. La hipófisis epitelial se desarrolla a expensas del ectodermo, diferenciándose en tres lóbulos: la parte anterior propia, la parte intermedia y la parte tuberosa. La parte anterior propia o lóbulo anterior propio, forma la mayor parte de la glándula y viene a rodear las superficies caudal y ventral del infundíbulo. La parte intermedia se desarrolla a expensas del extremo dorso-caudal del fundamento hipofisario primitivo. En su posición adulta está situada posteriormente al lóbulo neural, penetrando entre este último y el lóbulo anterior. La parte tuberosa procede de un par de procesos que crecen hacia delante desde el resto de la glándula. Estos procesos no se separan para formar placas epiteliales separadas como sucede los anuros, sino que conservan su relación con el lóbulo anterior durante la vida del individuo.

En *Necturus* y *Amphiuma* el lóbulo anterior está considerablemente dilatado. A juzgar por estos hechos solamente, estas dos formas son las primitivas en los anfibios caudados y están bastante íntimamente relacionadas con ciertos peces.

Translation by José F. Nonidez
Cornell Medical College, New York

THE MORPHOGENESIS OF THE HYPOPHYSIS IN THE TAILED AMPHIBIA

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NINETEEN FIGURES

The pars tuberalis has been recognized as a division of the epithelial hypophysis quite distinct from either the anterior lobe proper or the pars intermedia only within the past few years. At present the developmental and adult relations of the pars tuberalis are well known for a number of the vertebrate classes, viz., mammals, birds, reptiles, and to a certain extent for the amphibia, due to the researches of Tilney, Bolk, Woerdeman, Baumgartner, Parker, the writer, and others. The question as to the presence of an homologous lobe in the hypophysis of the remaining vertebrates is one which must be settled before broad generalizations may be made with assurance. The homologies attempted by Woerdeman, for example, seemed premature, since he did not have at his command the developmental histories of the gland for the amphibia or the teleost fishes.

In a previous paper (Atwell, '18 a) the writer has described the development of the hypophysis in the anura. In these forms the pars tuberalis is developed as a pair of buds which grow nasalward and, at about the time of metamorphosis, become detached from the remainder of the epithelial portion to form two discrete epithelial plaques. A preliminary study of the development of the gland in the tailed amphibia showed certain differences to exist, one in particular being of sufficient interest to warrant a more detailed investigation.

PLAN AND METHODS

It has been the plan of the present study to follow the development of the hypophysis by close stages in one genus of the urodeles and then to compare with this certain larval and adult

stages from other of the tailed amphibia. For the former *Ambystoma* was chosen. Fertilized eggs of the species *A. punctatum* were collected and embryos and larvae taken and preserved at frequent intervals. Older specimens were of *A. punctatum*, *A. tigrinum*, and *A. jeffersonianum*. For comparison, larval and adult stages of *Necturus maculosus*, *Spelerpes bislineatus*, and *Amphiuma* means were studied.

The fixative most often employed was Bouin's fluid, but for certain of the younger and a few of the older stages formol-bichromate or corrosive sublimate was used. In the case of the *Amphiuma*, vascular injection was employed in fixation, preceded by physiological saline solutions to remove the blood corpuscles. Series of sections prepared and utilized for this study total twenty-eight series of *Ambystoma*, one of *Necturus*, three of *Spelerpes*, and two of *Amphiuma*. Free use was made of the Born wax-plate method of reconstruction to demonstrate the successive stages in the morphogenesis of the gland. Models were constructed at a magnification of 200 diameters for the larval stages and of 100 or, in one case, 50 diameters, for the adult stages. The drawings were made at the original magnification and have been reduced in most cases one-half off. The magnification given with the figures indicates their present actual size.

Acknowledgment is due to Miss Ida Sitler, formerly of Smith College, now of Hollin's College, Virginia, for the preparation of a number of the wax-plate reconstructions. I wish also to express my sincere thanks and appreciation to Mrs. H. H. Wilder, of Smith College, for the specimens of *Spelerpes bislineatus* which she kindly collected and fixed for me; likewise to Prof. Irving Hardesty for suggestions and aid in securing the specimens of *Amphiuma*.

OBSERVATIONS

a. Development of the hypophysis in Ambystoma

4 to 6.5-mm. embryos of Ambystoma punctatum. At the 4-mm. stage the hypophysis fundamēt is already well formed. In sagittal sections it is evident as a solid, wedge-shaped mass

of cells extending dorsalward from the inner layer of the ectoderm at the cranial end of the oral pit. It lies between the wall of the neural tube and the foregut.

By the time the embryo has attained a length of 5.5 mm. the hypophysis has become more elongated and is more tightly wedged between the brain wall and the foregut. Mitotic figures are present and the total number of cells composing the gland has increased considerably. Neither this stage nor the preceding gives satisfactory evidence to confirm a possible bilateral origin for the gland such as has been described by Kingsley and Thyng.

A 6.5-mm. embryo shows the gland united to the ectoderm by a stalwart connection. The oral plate is intact.

7.5-mm. embryo of A. punctatum. An approximately mid-sagittal section of the hypophysis region from a 7.5-mm. embryo is shown in figure 1. The epithelial stalk, by which the gland maintains its attachment to the ectoderm, has become a slender cord of cells. The dorsal end of the gland is enlarged and club-like. It presses tightly against the caudal termination of the diencephalic wall. The notochord, at this stage, does not extend so far cephalad as in a corresponding stage in the frog tadpole, and consequently its cephalic end is not in intimate relation with either the infundibulum or the epithelial hypophysis. The oral plate is intact.

9- and 10-mm. larvae of A. punctatum. Figure 2 A shows a wax-plate reconstruction of the hypophysis from a 9-mm. larva viewed from the ventral surface, with the caudal end below. The gland is just losing its connection with the ectoderm as its drawn-out cephalic end indicates. The detachment takes place close to the gland in such a manner that a considerable stalk is left attached to the epithelium. This is apparently retracted into the epithelium or else completely disappears. I have never noted in the Amphibia a separation of the stalk close to the ectoderm with the consequent formation of remnants, such as frequently are seen in mammals, and which may give rise to a 'pharyngeal hypophysis' or a 'parahypophysis.' There is a certain rearrangement, with more compact grouping, of the cells

which are to form the pars intermedia. These are situated at the caudal free extremity of the gland.

12-mm. larva of A. punctatum. A wax-plate reconstruction from a larva of this stage is shown in figure 2 B. Comparison of A and B of figure 2 shows that after the detachment of the gland

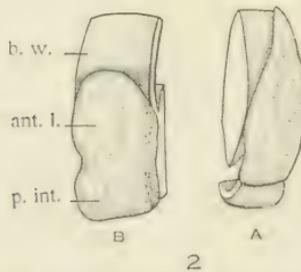
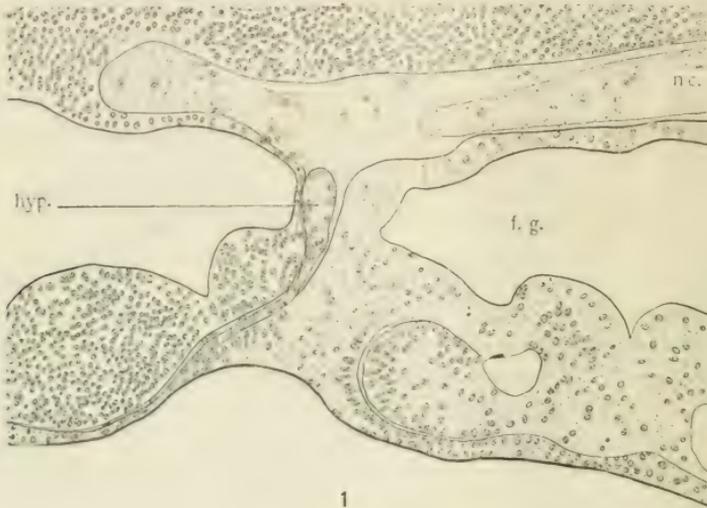


Fig. 1 Approximate midsagittal section of hypophysis region from 7.5-mm. larva of *Ambystoma*; nasal end at left. *hyp.*, epithelial hypophysis; *f.g.*, foregut; *nc.*, notochord. $\times 100$.

Fig. 2 Wax-plate reconstructions of the epithelial hypophysis and adjacent brain wall viewed from the ventral surface. *A*, from a 9-mm. larva of *Ambystoma*; *B*, from a 12-mm. larva. Caudal end below. *b.w.*, brain wall; *ant.l.*, anterior lobe; *p.int.*, pars intermedia. $\times 100$.

from the epithelium the hypophysis becomes both relatively and absolutely shorter. The cephalic end of the gland becomes blunt and round. It appears as though some traction which previously had kept the gland drawn out and elongated had been rather suddenly relieved. The pars intermedia is more distinctly differentiated and its separation from the anterior lobe proper is indicated by grooves at the sides.

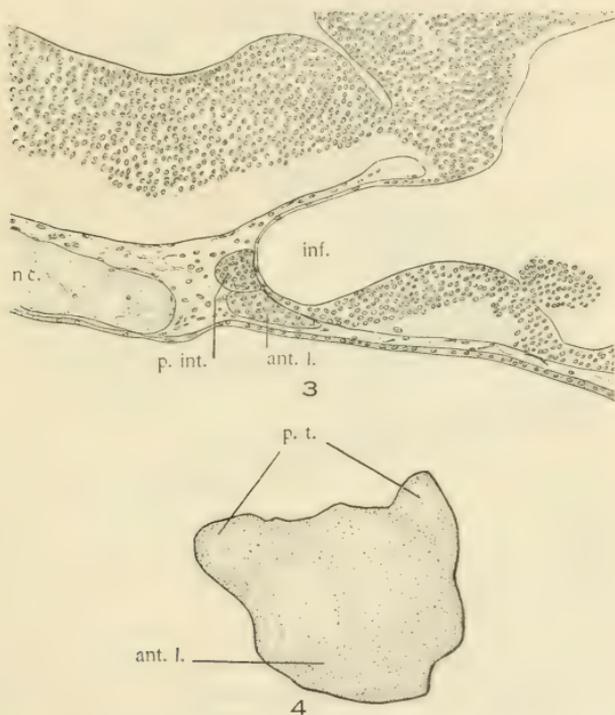


Fig. 3 Sagittal section of hypophysis region, 15-mm. *Ambystoma* larva. Nasal end at right. *nc.*, notochord; *inf.*, infundibulum; *ant.l.*, anterior lobe; *p.int.*, pars intermedia. $\times 100$.

Fig. 4 Ventral view of a wax-plate reconstruction of the epithelial hypophysis from a 38-mm. *Ambystoma* larva; caudal end below. *p.t.*, pars tuberalis; *ant.l.*, anterior lobe. $\times 100$.

14- and 15-mm. larvae of A. punctatum. Between the 12-mm. and 14-mm. stages the rupture of the oral plate occurs. Transverse sections of the hypophysis of 14-mm. larvae show a thin shelf-like lateral extension on each side of the anterior lobe proper. These are doubtless the lateral lobes which later form the pars tuberalis. A midsagittal section of the hypophysis region from a 15-mm. larva is given in figure 3.

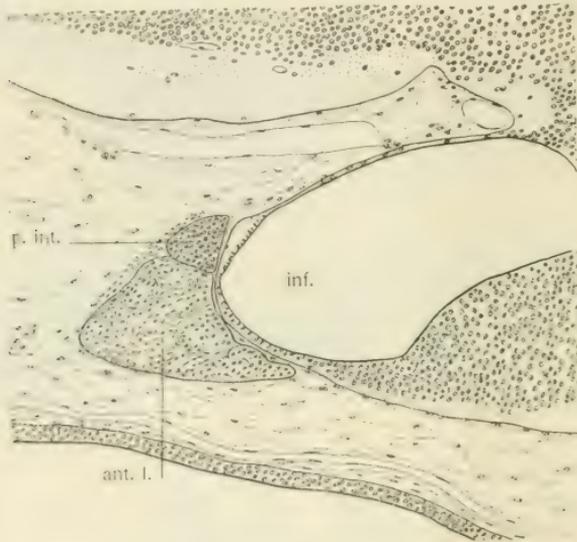


Fig. 5 Midsagittal section of the hypophysis region from a 40-mm. *Ambystoma* larva; nasal end at right. *inf.*, infundibulum; *p. int.*, pars intermedia; *ant. l.*, anterior lobe. $\times 100$.

38-, 40-, and 60-mm. larvae of Ambystoma. Figure 4 shows a ventral view of a wax-plate reconstruction of the epithelial hypophysis from a 38-mm. larva. The two buds which compose the pars tuberalis are well differentiated. The pars intermedia cannot be seen from this surface. The reason for this is well illustrated by figure 5, which gives a midsagittal section from a 40-mm. larva. The anterior lobe is molded around the caudal end of the infundibulum in such a manner that, with

the pars intermedia, a cup-shaped structure is formed. The pars intermedia is situated dorsal to the anterior lobe and at some distance forward from its caudal extremity. The neural lobe is apparent as a thickening of the part of the infundibular wall adjacent to the pars intermedia. The two bud-like portions of the pars tuberalis grow forward, becoming more elongated (fig. 6). The 60-mm. larva shows a considerable increase

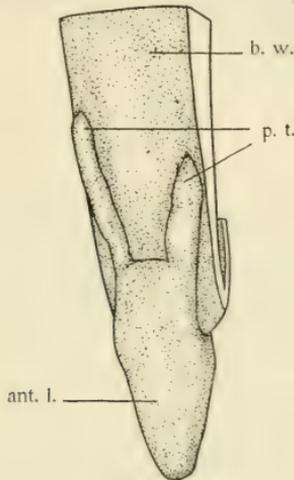


Fig. 6 Ventral view of a wax-plate reconstruction of the hypophysis from a 40-mm. larva of *Ambystoma*. Caudal end is below. *b.w.*, brain wall; *p.t.*, pars tuberalis; *ant.l.*, anterior lobe. $\times 100$.

in the size of all parts of the gland, but the enlargement of the anterior lobe proper is especially apparent.

Adult Ambystoma jeffersonianum. The hypophysis of the adult ambystoma is shown in sagittal section in figure 7. Three views of the wax-plate reconstruction are given in figures 8, 9, and 10. The anterior lobe proper is seen to be the most caudal portion of the gland. The name 'anterior lobe,' therefore, has not been applied because of the relative position of the part in the adult amphibian, but because of its homology to the corresponding lobe in the higher vertebrates. Both sections and

reconstructions show that the pars intermedia is small. Its greatest dimension, which is from side to side, is less than the width of the anterior lobe proper. It is located dorsally and lies wedged in between the neural lobe and the anterior lobe (figs. 7, 8, and 9).

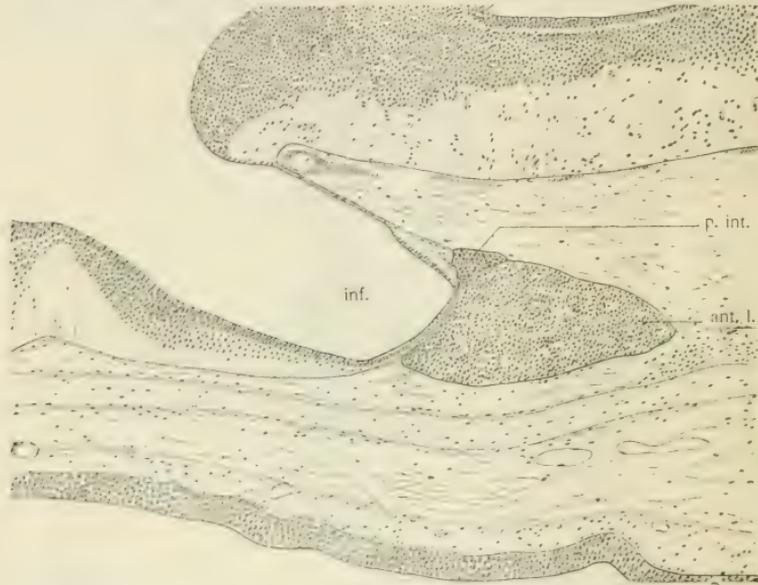
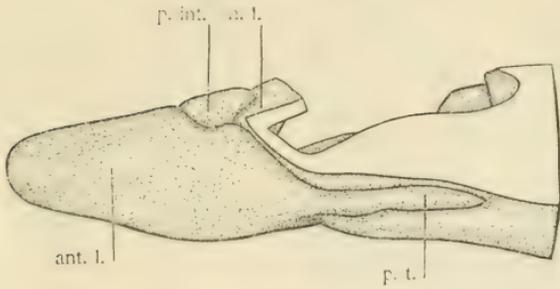
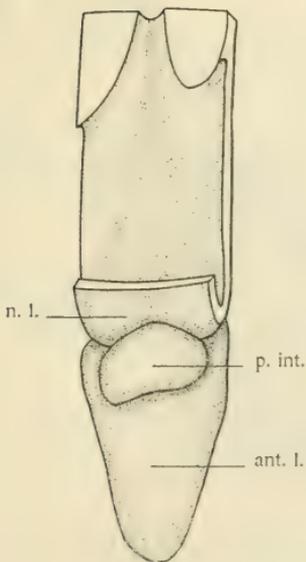


Fig. 7. Midsagittal section of the hypophysis region of an adult *Ambystoma*. The nasal end is at the left. *inf.*, infundibulum; *p. int.*, pars intermedia; *ant. l.*, anterior lobe. $\times 40$.

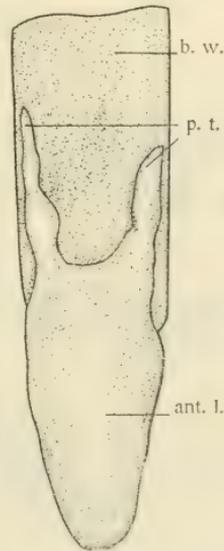
The adult condition of the pars tuberalis resembles very closely the larval. As shown by figures 8 and 10, the two processes of the pars tuberalis maintain their connection with the main body of the gland. They are elongated and lie embedded in the pia mater close to the floor of the brain. These processes do not become detached, as in the frog and the toad, to form separate epithelial discs, or plaques, but remain throughout adult life as two tongue-like processes extending nasalward from the anterior lobe proper.



8



9



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Figs. 8, 9, and 10 Wax-plate reconstruction of hypophysis and adjacent brain wall of an adult *Ambystoma*. Figure 8 views it from the right side; figure 9 is a dorsal view; figure 10 is a ventral view, caudal end below. *n.l.*, neural lobe; *p.int.*, pars intermedia; *p.t.*, pars tuberalis; *ant.l.*, anterior lobe proper. $\times 50$.

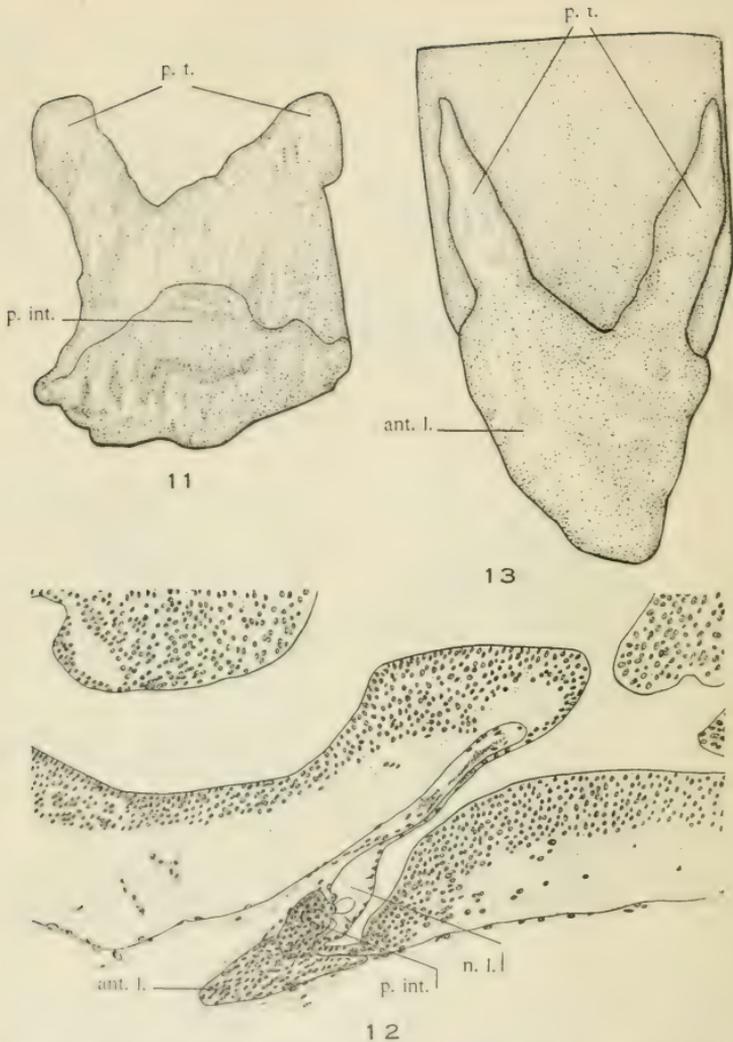


Fig. 11 Dorsal view of a wax-plate reconstruction of the epithelial hypophysis from a 19.5-mm. *Spelerpes* larva, caudal end below. *p.t.*, pars tuberalis; *p.int.*, pars intermedia. $\times 100$.

Fig. 12 Midsagittal section of the hypophysis region from an adult of *Spelerpes bislineatus*, nasal end at right. *ant.l.*, anterior lobe proper; *p.int.*, pars intermedia; *n.l.*, neural lobe. $\times 100$.

Fig. 13 Wax-plate reconstruction of the hypophysis of an adult *Spelerpes bislineatus*; caudal end below. *p.t.*, pars tuberalis; *ant.l.*, anterior lobe proper. $\times 100$.

b. The hypophysis of Spelerpes

For comparison the hypophysis was studied in three other tailed amphibia, with special attention given to the adult morphology of the gland. The forms chosen were *Spelerpes bislineatus*, *Necturus maculosus*; and *Amphiuma means*.

The specimens of *Spelerpes* include a 19.5-mm. larva, a larva of the 'pre-metamorphic stage' (Wilder), and an adult. A dorsal view of a wax-plate reconstruction of the epithelial hypophysis from the younger larva is shown in figure 11 and from

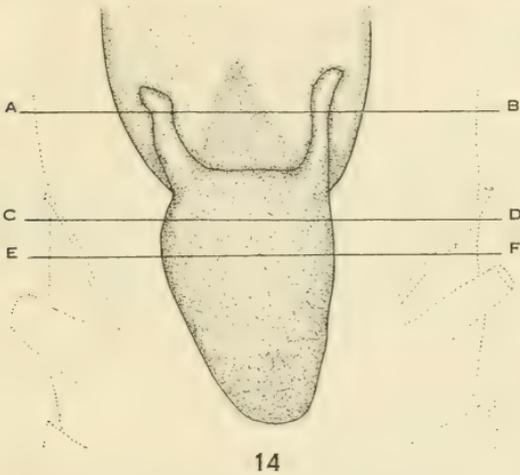


Fig. 14 Camera-lucida drawing showing a ventral view of the hypophysis of an adult of *Necturus maculosus*, caudal end below. *A-B*, *C-D*, and *E-F*, indicate planes of sections shown in figures 15, 16, and 17, respectively. $\times 15$.

the adult in figure 13. Figure 12 presents a sagittal section of the adult hypophysis. It may be seen that the pars intermedia is relatively large. The anterior lobe proper is small and is situated almost entirely caudal to the infundibulum. Corresponding to the large size of the pars intermedia the neural lobe is also relatively large. The two components of the pars tuberalis maintain their attachment to the anterior lobe proper, even in the adult stage (fig. 13).

c. The hypophysis of Necturus

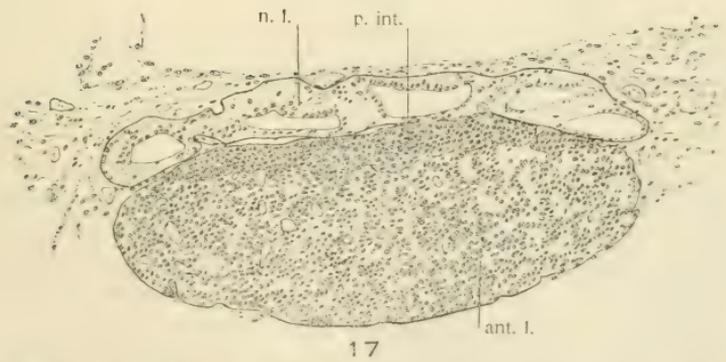
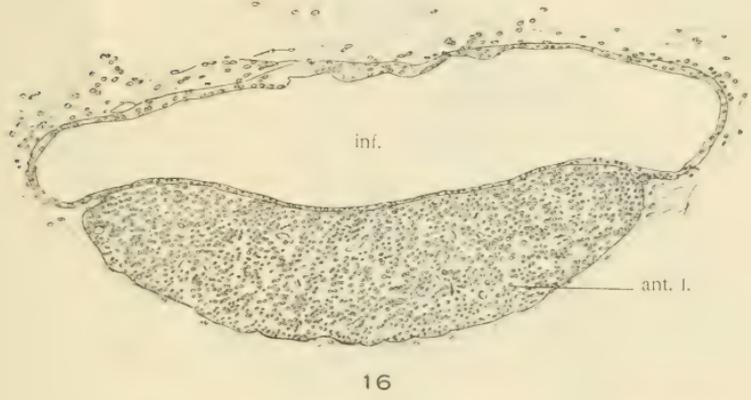
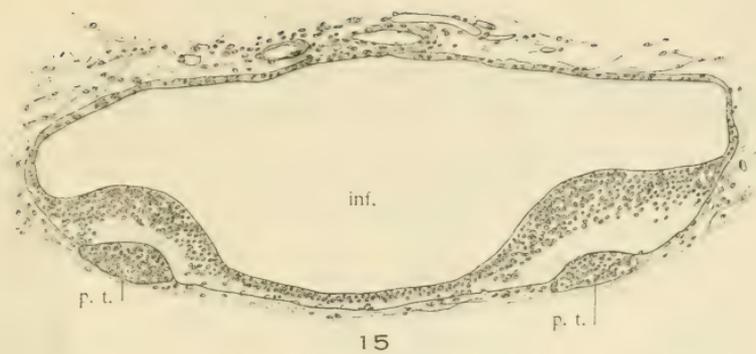
A camera-lucida sketch of the hypophysis and adjacent brain floor in an adult is given in figure 14. The lines *A-B*, *C-D*, and *E-F* indicate the positions of the transverse sections shown in figures 15, 16, and 17, respectively. As may be seen from figure 14, the two portions of the pars tuberalis remain united to the anterior lobe. The latter is elongated with its greatest dimension extending anteroposteriorly.

Figure 15 shows a transverse section through the pars tuberalis (fig. 14, *A-B*), which is seen as two epithelial strands lying under thickenings in the floor of the diencephalon. Between these two thickenings the infundibular floor is very thin. A more caudal section is shown in figure 16 (fig. 14, *C-D*). Here only the thin-walled infundibulum and the anterior lobe proper may be seen. A section somewhat farther caudalward is given in figure 17. The neural lobe, the pars intermedia, and the anterior lobe proper are seen. The neural lobe is very much sacculated, so that the infundibular cavity is cut five times in this one section. The pars intermedia is a thin strip situated between the other two parts. It is not very vascular in comparison with the anterior lobe: its nuclei are more closely crowded together, and with the commoner stains it is considerably darker in appearance. The anterior lobe contains large, thin-walled blood spaces and its cells have a cord-like arrangement.

d. The hypophysis of Amphiuma

Sections and a wax-plate reconstruction from adults of *Amphiuma* means (figs. 18 and 19) show the same general features as seen in the other tailed amphibia studied. Certain distinguishing features may be noted, however. The gland appears to be flattened from side to side (fig. 18) and much thicker in a dorsoventral dimension (fig. 19). In the latter respect the difference is marked when compared with *Spelerpes*. Also in *Amphiuma* the anterior lobe is relatively greater in bulk.

The pars intermedia is compact in structure and dark staining (fig. 19). The neural lobe is well sacculated, as in *Necturus*.



Figs. 15, 16, and 17 Transverse sections of the hypophysis of an adult *Necurus*, shown in the gross in figure 14. The planes of the sections are shown by the lines A-B, C-D, E-F, figure 14, respectively. $\times 50$.

The pars tuberalis, as in the other forms studied, maintains its connection with the nasal end of the anterior lobe (fig. 18).



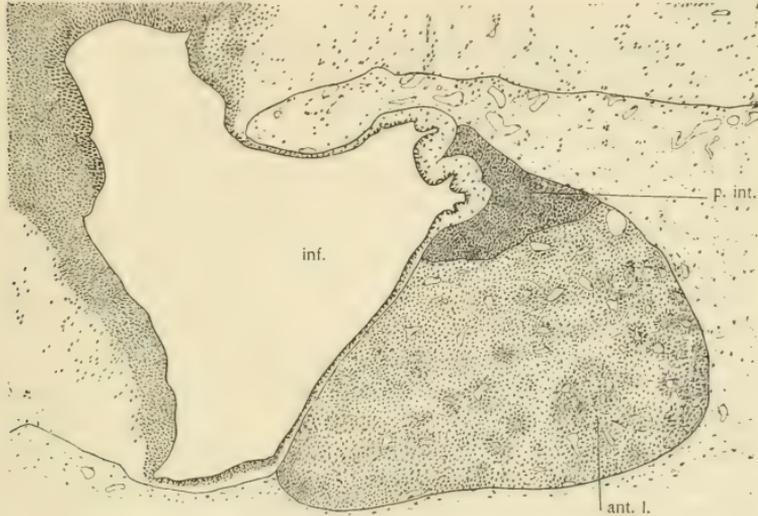
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Fig. 18 Ventral view of a wax-plate reconstruction of the hypophysis of an adult *Amphiuma*, caudal end below. *b.w.*, brain wall; *p.t.*, pars tuberalis; *ant.l.*, anterior lobe proper. $\times 25$.

DISCUSSION

This study has not been primarily concerned with the early stages in the development of the hypophysis. All the evidence, however, goes to confirm the opinion that the gland is entirely ectodermal in origin, and that no contribution is made by the entoderm or the notochord, as has sometimes been claimed for the amphibia (Kupffer, '94, and Valenti, '95). The material studied has not given satisfactory evidence to confirm the statement of Kingsley and Thyng ('04) that the hypophysis has a bilateral origin in *Ambystoma*.

The hypophysis breaks loose from the ectoderm in amphibia a considerable time before the rupture of the oral plate. This is the reverse order from that obtaining in birds and mammals where the oral plate ruptures early and the hypophysis maintains its connection with the ectoderm until much later.



19

Fig. 19 Midsagittal section of the hypophysis region of an adult *Amphiuma*. Nasal end at left. *inf.*, infundibulum; *p.int.*, pars intermedia; *ant.l.*, anterior lobe proper. $\times 30$.

That the two tongue-like processes attached to the nasal end of the anterior lobe are to be considered homologous with the pars tuberalis in the higher vertebrates seems to me very certain. They are similar in all respects to the processes seen in the larval stages of the anura (cf. Atwell, '18 a).

These structures have been noted by Stendell ('14), who describes them in these words:

Bei den Urodelen und Anuren kann noch ein besonderer Hypophysenteil unterschieden werden, der hier Erwähnung finden möge.

Er liegt im allgemeinen von den übrigen Hypophysenabschnitten, dem Haupt- und Zwischenlappen, getrennt weiter vorn fast unter dem Chiasma opticum, dem Hirnboden dicht angepresst (Fig. 43, 44 und 26c). Er ist durchaus drüsiger Natur und zum Darmteil gehörig. Meistens ist er paarig entwickelt in Form zweier symmetrisch zu beiden Seiten der Medianen gelegener, flach-linsenförmiger Zellhäufchen. Dieser Hypophysenteil ist Pars anterior, auch Pars chiasmatic genannt worden.

Stendell is uncertain whether this pair of processes should be classed with the anterior lobe proper or the pars intermedia. He has not attempted to homologize it with the pars tuberalis, since the individuality of this lobe was not recognized by him for any of the vertebrate classes.

The most striking feature to be observed in comparing the hypophysis of the urodeles and the anura is that in the former the two tongue-like processes of the pars tuberalis do not become detached from the anterior lobe proper, even in adult life. It will be recalled that in the anura the processes become detached at the time of metamorphosis and form two discrete epithelial plaques. This peculiarity was noted by Stendell for at least one form. He states: "Ferner scheint die Hypophyse von *Proteus anguineus* in dieser Beziehung primitive Verhältnisse zu zeigen. Bei ihr nämlich bleibt jener vordere Teil zeitlebens als zungenförmige Verlängerung an dem Hauptlappen des Darmteils hängen."

That this condition is a constant one for the tailed amphibia seems certain, since it is also found to obtain in *Ambystoma*, *Spelerpes*, *Necturus*, and *Amphiuma*. It is interesting to speculate upon a possible relationship between this apparently 'primitive' condition of the hypophysis and the retention of the tail throughout adult life. Is there anything more than coincidence to be assigned to the fact that in the higher Amphibia when the tail is lost at metamorphosis, the pars tuberalis likewise becomes detached?

It must be noted that the peculiar detached condition of the pars tuberalis in the adult anura is not general for the higher vertebrate classes, since in birds and mammals the lobe is usually found connected with the remainder of the gland. It is

often detached, however, and sometimes disappears entirely in certain reptiles (Baumgartner, '16).

The sacculations of the neural lobe, noted in two of the forms studied, were not observed for the frog or the toad (Atwell, '18 a). In this respect the hypophysis of the tailed amphibia is the more closely related to that of the fishes, where in certain forms, notably the elasmobranchs, the sacculation of the neural lobe and its interdigitation with the pars intermedia are very extensive.

SUMMARY

In the tailed amphibia the epithelial hypophysis is developed from the ectoderm and differentiates into three lobes: the pars anterior proprior, the pars intermedia, and the pars tuberalis. The pars anterior proprior, or anterior lobe proper, forms the main bulk of the gland and comes to lie caudal and ventral to the infundibulum. The pars intermedia is developed from the dorsocaudal extremity of the early hypophysial fundament. In its adult position it lies caudal to the neural lobe and dorsal to the anterior lobe. The pars tuberalis develops from a pair of processes which grow forward from the remainder of the gland. These processes do not become detached to form separate epithelial plaques as in the anura, but maintain their connections with the anterior lobe throughout life. The neural lobe is considerably sacculated in *Necturus* and *Amphiuma*. Judging from this criterion alone, these two forms are primitive and are rather closely related to certain of the fishes.

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