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THE DEVELOPMENT OF THE RECTUM IN THE HUMAN EMBRYO

FRANKLIN PARADISE JOHNSON

The Anatomical Laboratory of the University of Missouri

TWENTY-FIVE FIGURES

INTRODUCTION

This is the third of a series of papers concerning the development of the mucous membrane of the digestive tract. The first two (Johnson '10 and '13) deal with the oesophagus, stomach, and small and large intestines. In this paper an account of the development of the rectum is given, particular attention being paid to the formation of the pars analis recti.

The upper portion of the rectum, that is, that part which goes into the formation of the ampulla recti, develops in a similar manner to the colon. In this region, ridges, folds, villi, and glands make their appearance in the order named, and, simultaneously with the further development of glands, the villi disappear. As these changes have already been described by stages, another account need not be given here. Attention, however, might again be called to the fact that the rectum is more precocious in its development than the other parts of the large intestine; thus we find in the rectum conditions which are always in advance of those higher up. It is to be noted also that the ridges and folds of the mucous membrane which precede the formation of glands and villi, at the level where these pass over into the anal region, are not longitudinal in direction, as found in the remaining portions of the large gut, but are transverse. In still another respect the mucous membrane of the ampulla recti of the embryo differs from that of the colon. One finds numerous epithelial

gland cysts, which exist throughout a considerably long period of embryonic life, but which have disappeared before birth. These also have been described in the former paper.

Regarding the material used and the method of study employed very little need be said. Whole embryos, sectioned serially, have been used for stages up to 44.3 mm. From this stage upward, with the exception of an embryo of 65 mm. which was also sectioned whole, the pars analis recti, including a portion of the ampulla recti and the skin around the anus, was cut out of the embryos and made into serial sections, some cross, and some longitudinal. The serial sections of the whole embryos obtained from the Harvard Embryological Collection are designated, H.E.C.; those from the collection of the University of Missouri, H.

Wax models have been made to illustrate the conditions at various stages of development. Use was also made of gross dissections, and particularly of mid-sagittal sections through the whole pelvis. The models of the epithelial tube, except in case of older stages, have been made at a magnification of 145 diameters, and therefore allow easy comparison with those made of other portions of the digestive tube.

A difficulty which presented itself at the outset of this work was one concerning terminology. One finds in the literature varying descriptions of the rectum; the same terms being used to denote different portions. It was necessary, therefore, to review the literature concerning the anatomy of the adult rectum and determine upon the most generally accepted terminology. A brief review of the literature concerning the limits and subdivisions of the rectum is first given. To it are added certain topographical relations as described by various authors, for comparison with the observations noted in the section on developmental topography included in this paper.

Although but few observations have been made regarding the development of the plicae transversales recti, it was thought best for the sake of completeness, as well as for comparison with the observations made on the larger folds of the mucous membrane found in the small and large intestines, to include a brief descrip-

tion of them here. The literature regarding those of the adult, therefore, is briefly alluded to.

More attention, however, has been paid to the investigations bearing upon the embryology and histology of the pars analis recti. More complete reviews of the literature on this subject have been given by Braun ('01) and Zimmermann ('04). The following résumé of the literature is taken up under separate headings.

LITERATURE

Boundaries and subdivisions of the rectum

According to Merkel, Sanson in 1817 described the rectum as beginning at the pelvic brim in the region of the sacro-iliac synchondrosis. The first of its three divisions, as described by Sanson, passes obliquely downwards from left to right, in which it describes a slight curve toward the right and finally reaches the mid-line against the third sacral vertebra. It is completely surrounded by a peritoneal fold called the mesorectum. The second part extends along the concavity of the sacrum to the end of the coccyx, where the third part begins. This turns backwards and ends in the anus. This description was accepted and used by most of the anatomists up until 1885 when Treves (according to Symington) modified it. Treves considered as the rectum only that part of the large intestine which is devoid of mesentery, which he says, begins at the level of the third sacral vertebra. That portion of the rectum previously known as the first part of the rectum he includes with the sigmoid flexure under the term 'sigmoid' or 'omega loop.'

Symington ('88) accepted this view of Treves in regard to the upper limit of the rectum, but regards only the second part of the rectum of the older anatomists as the rectum proper. The third part he calls the 'anal canal,' which he says, had previously been incorrectly described. It differs from the rectum in that it "exists normally as a longitudinal fissure." It is about an inch in length.

Merkel ('00) also regards the termination of the mesocolon as the upper limit of the rectum, but divides it into two parts. The

upper he calls the "pars ampullaris recti;" the lower, "the pars analis recti." He says:

Zu dieser wichtigen Änderung im Verhalten des Bauchfelles kommen noch zwei Punkte in der Structur der Darmwand selbst, welche dem untersten Darmstück eine besondere Stellung anweisen, erstens die beträchtliche Verstärkung der Muskulatur und zweitens die Neuordnung der Langfäaserschichte (Aufhören der Tänien). Dieses Verhalten der Muskulatur ordnet das Rektum in die Reihe der Blasenbehälter ein, als Kotblase (Waldeyer), eine Annahme, welche durch häufig zu beobachtende spindelförmig Erweiterung des Darmrohres (Pars ampullaris recti) (Pars pelvina, Waldeyer) gestützt wird. Dieser erste Teil des Mastdarmes reicht aber niemals bis zur Afteröffnung hin, sondern man findet das letzte Stück immer, in gleicher Weise leer and zusammengezogen, so dass man dieselbe als zweiten Teil des Mastdarmes (als Pars analis recti) (Canalis analis, Symington) von der Pars ampullaris zu trennen hat.

In a paper regarding this subject, Paterson ('08) writes: "The superior rectal valve, though variable in its form, is, in my opinion so generally present that it may be looked upon as the true junction of the pelvic colon and the rectum."

Symington ('12) believes with Paterson that the superior rectal valve is a more reliable guide to the colo-rectal junction than the termination of the mesocolon, as this latter ends so gradually: "There is no reliable bony landmark serving as a guide to the colo-rectal junction, since while the empty and contracted rectum usually starts opposite the third sacral vertebra, when the rectum is distended this junction may rise as high or even higher than the sacral promontory."

Of the modern text-books of anatomy, Sobotta-McMurrich is the only one of those I have examined which still follows the description of Sanson, that is, considers three divisions of the rectum, the first of which begins at the sacral promontory. Cunningham regards as the rectum only that part of the digestive tube which lies between the third sacral vertebra and the pelvic floor. The 'anal canal' is treated separately. Other authors speak of two parts which lie between the third sacral vertebra or termination of the mesocolon, the pelvic floor or diaphragm, and the anus. The terminology, however, differs considerably. Thus the ampulla is considered as the lower part of the first portion of

the rectum by Testut, Kopsch-Rauber, and Spalteholz. The terms 'pars pelvina' and 'pars perinealis' are employed for the two parts of the rectum by Waldeyer in Joessel's Lehrbuch. Poirier et Charpy use the terms 'rectum pelvien' and 'rectum perineal.' Morris and Gray apply no especial name to the first part. The former calls the second part the 'anal canal,' the latter the 'inferior' or 'prostatic' portion. Gray, like Spalteholz, Kopsch-Rauber, and others, regards only the lower widened portion of the first part as the ampulla recti. Corning considers as synonyms for the first part of the rectum the terms *pars ampullaris recti*, *pars pelvina*, and *flexura sacralis*, while for the second part, *pars analis recti*, *pars perinealis*, and *flexura perinealis*; and says that the three sets of terms are applicable from the standpoints of physiology, topographic anatomy, and descriptive anatomy, respectively.

Likewise in the case of boundary lines between the *pars analis recti* and the ampulla recti one finds varying descriptions. Thus, the tip of the coccyx, the pelvic diaphragm, and the beginning of the pelvic floor are all given. It has also been described merely as the beginning of the narrower portion found below the widened ampulla. Still another line of division is given as the ano-rectal line, the point at which the simple columnar epithelium leaves off and a stratified columnar epithelium begins. However, these points do not exactly coincide. The ano-rectal line, even in late fetal stages, does not correspond to the beginning of the narrow portion of the rectum; while the statement that it ends at the tip of the coccyx is indefinite.

The terms as found in the B.N.A. list are used consistently throughout this paper whenever possible. As regards the meaning of the term 'ampulla recti,' the work of Merkel and Corning is followed, designating the whole of the first portion of the rectum. By 'pars analis recti' will be meant that portion which has, under ordinary conditions a much narrower caliber than the ampulla. This portion which develops from a definite embryonic structure, does not have as its upper limit the histological ano-rectal line. Its upper boundary corresponds fairly closely, however, with the beginning of the pelvic floor.

Plicae transversales recti

Although the plicae transversales recti (valves of Houston), which are found in the ampulla recti, have attracted a great deal of attention from many anatomists, only a brief reference to the literature regarding them need be made in this paper. Most anatomists agree that Houston, after whom the plicae were named, was the first to describe them adequately. The name Kohlrausch, however, is often used in connection with the lower of these folds.

Baur ('60) made a careful study of the plicae and found that three are usually present. He also found indications of them in embryos of three, three to four, and four to five months, and at birth.

Vance ('78) describes these folds in a number of adult subjects and emphasizes particularly the spiral arrangement of some of them. He asks: "Is it not reasonable to suppose that both spiral folds and independent valves exist in the human rectum as rudiments, and that, when present in that organ, they must be looked upon as illustrations of the law of reversion?" In just what manner they are to be regarded as illustrations of this law, Vance does not state.

Otis ('87) finds the rectal plicae constantly present and states that there are either two or three of them, two of which are constant in position, and one of which is variable.

Pennington ('00) attaches particular importance to the folds from a clinical point of view, stating that they are often the cause of constipation. In describing their histological structure he states that some are composed of the mucosa and the submucosa alone, some have the circular muscle coat involved, while still others have a part or the whole of the longitudinal muscle coat.

Rectal columns, sinuses and valves

The longitudinal folds at the lower extremity of the rectum known as the columnae recti (columns of Morgagni) vary not only in size and number, but also in position. This conclusion is based not only upon the specimens I have myself examined, but upon the widely varying descriptions found in literature. Thus

Ball ('94) describes the columns as lying wholly within the ampulla, while on the contrary, Birmingham (in Cunningham's Text-book of Anatomy) shows them as lying wholly within the pars analis recti. Most authors, however, figure them as lying partly within the two portions of the rectum.

The same is true in regard to the descriptions of the valves of Morgagni and rectal sinuses (sinus rectales). Some authors show them as very definite structures, closely resembling the valves of the aorta, with wide pockets behind and arranged regularly in between the bases of the rectal columns. Other authors state that they are inconstant and vary greatly in size and shape, and figure them as narrow clefts in between two adjacent columns.

The rectal columns and valves according to Bodenhamer ('88) were well understood and correctly described by Morgagni and Glisson, and by other early anatomists.

Bodenhamer gives the credit to Glisson for the discovery of the columns, valves and sinuses. Braun ('01), on the other hand, while admitting that Glisson discovered the valves, states that the columns and 'Sinuositäten' (meaning the smaller secondary sinuses) were discovered and described by Morgagni (1719). Braun further states that Heister (1727) first described the transition zone between the mucous membrane of the rectum and the outer integument.

Chiari ('78) describes the rectal sinuses under normal and pathological conditions. He believes that by an outward and downward extension of these structures, fistulae are produced, and in support of his theory he gives several figures of the sinuses in different stages of fistulae formation. The same view is held by Bodenhamer ('88).

The first noteworthy attempt to describe accurately the mucous membrane of the anal region was made by Robin and Cadat in 1874. In describing the junction of the ampulla and the pars analis recti, they state that the latter differs from the former only in that it is not provided with the intestinal glands. The upper limit of the pars analis recti is described as the line where the intestinal glands leave off ('ligne ano-rectale'), while the lower limit, the 'ligne sinueuse' lies at a distance of 5 to 8 mm. below

the ano-rectal line. The zone marked off between these two lines is broken and raised from place to place by the columns of Morgagni, between which are found the intercolumnar pits or depressions ('godets'). The semilunar valves are found at the inferior extremity of this zone. Two types of epithelia are present, but it is not clear from the descriptions just where these are to be found. In an accompanying figure, the limits of the two epithelia are definitely shown and the following description is based largely upon the figure and its accompanying legend. The first is the prismatic type of epithelium which, like that of the colon, extends from the ano-rectal line to the bottoms of the sinus-like depressions. The second, the stratified squamous type, lines the internal wall of the sinus, that is, the supero-external wall of the valve. Below the sinuous or "true anal line" is described a region which is different from the skin proper, but which takes on more and more the structure of the skin when followed caudally.

This region which is termed the "zone cutanée anale lisse," extends down to where the hairs begin, a distance of 12 to 20 mm. below the sinuous line. It differs from the skin in that its cells are without pigment, only slightly cornified, the dermal papillae are small and few, and sebaceous and sweat glands are lacking.

Herrmann and Defosses ('80) state that the mucosa of the inferior extremity of the rectum is not directly continuous with the external integument, but that there exists at this level a circular zone, 6 to 12 mm. in height, corresponding to the muscular columns of Morgagni, and which represents a persistent part of the cloaca of the embryo. On the columns the epithelium is stratified polygonal, with superficial plate-like cells, while in the depressions it is of the stratified prismatic type.

Herrmann ('80) states that the anal mucous membrane is limited by the 'ano-rectale' and 'ano-cutanée' lines. The first he describes the position of definitely enough when he says here end the intestinal glands and the simple columnar epithelium of the colon, but his statement that it is to be found at the level of the upper borders of the anal valves and rectal columns is not clear.

The 'ano-cutaneous' line is drawn at the point where the anal epithelium passes over the stratified squamous type (Robin and Cadiat's "ligne sinueuse"). Below this line is the zone "cutanée peri-anale" ("zone cutanée lisse" of Robin and Cadiat). In agreement with the descriptions set forth by Robin and Cadiat, Herrmann says this region is deprived of hairs, sebaceous and sweat glands. Its epithelium is stratified polyhedral, composed of six to eight layers of cells, and differs from that of the skin by the absence of indented cells and a cornified covering, for although the superficial cells are somewhat flattened, they have not a lamellar form. The nuclei are clear and the cell bodies finely granular and stain the same as the underlying cells. Neither a stratum corneum nor stratum lucidum is present.

The anal mucous membrane proper, that is, the zone between the ano-rectal and ano-cutaneous lines, is thrown into folds (columns of Morgagni) between which lie rounded sinuses. Extending outward from these sinuses are recesses of a second type, narrow slit-like cavities which reach almost to the internal sphincter. A third type of sinus extends from the secondary type in the form of branched tubules. These, which he calls the "intramuscular sinuses" ("special acinose glands") are dealt with below. The anal mucosa is covered by an epithelium which is made up of several layers of polygonal cells but the superficial cells are of the prismatic type. The protoplasm of these prismatic cells is transparent and only slightly granular and not readily staining.

He states further that it is a general rule that the stratified polygonal epithelium covers the more elevated parts (columns of Morgagni), while the prismatic type is found in the depths of the mucosa, in other words, "those parts which are not exposed to pressure and mechanical effects."

Ball ('94) states that the "white line of Hilton" is the ano-rectal line of Herrmann, but since he says it occurs at the level of the semilunar valves, he undoubtedly means Herrmann's ano-cutaneous line. Birmingham ('09) uses the terms "white line of Hilton" and "ano-cutaneous line of Herrmann" synonymously.

Development of columns, sinuses and valves

Regarding the development of these structures very little has been written. The paper which deserves most credit is that of Herrmann ('80). His observations begin in an embryo of 85 mm. At this stage he states that the mucosa of the anal region forms a zone about 1 mm. in height, and contains on the sides excavations which are divided into several compartments by longitudinal folds. These folds of mucous membrane form the columns of Morgagni, and mark the places where the muscular columns are to develop. The epithelium is stratified prismatic, composed of two to three layers of cells, and is about 0.025 to 0.030 mm. thick. The peri-anal cutaneous zone is covered by a stratified squamous epithelium of six to eight layers of cells, but shows no traces of hairs or glands. At 140 mm. the longitudinal folds are more numerous and in places are no more than 0.1 mm. from the internal sphincter muscle. In the epithelium of the peri-anal zone, in its outer part, hair follicles and sebaceous glands are beginning to appear. Sweat glands are present as tortuous cylindrical tubes of epithelium which are greatly swollen at their extremities.

In an embryo of 190 mm. the muscularis mucosa is present in the region of the rectal ampulla, but is absent from the anal region. In neither the rectal or the anal mucosa are solitary nodules present. At 210 mm. the anal mucosa has grown to a height of 2.5 mm. and has an epithelium of 0.04 to 0.05 mm. in thickness. At birth the anal region has a height of 3.5 mm. The muscularis mucosae is completely formed and is prolonged in several strands which extend down into the rectal columns. Finally in an infant of two years, he says, are to be found the identical disposition of all structures which are found in the adult, only with reduced dimensions.

Ball ('94) regards the semilunar valves as vestigial remnants. He says: "I think it may with tolerable confidence be asserted that the anal valves are vestigial remains of the anal plate, the rest of which has disappeared in the process of development." No evidence, however, is given in support of this assumption.

Birmingham states that both the columns of Morgagni and the rectal valves are distinct in embryos of four to five, six and nine months.

Glands

Gay ('71) describes an elliptical ring of glands in the form of a wreath around the anus. The ring he states is 1.25 to 1.5 cm. broad and the inner border of the zone 1 to 1.5 cm. from the anal opening. These 'circum-anal' glands have the structure of sweat glands but they are considerably larger, being even larger than the axillary glands. They are entirely absent in between the ring and the anus where only sebaceous glands are to be found.

Robin and Cadiat ('74) describe the manner in which the intestinal glands leave off at the lower extremity of the rectum. When seen in longitudinal section, the last four to six intestinal glands are more closely packed together and their cells stain more intensely. They emphasize the fact that there is a smooth zone (the zone cutanée lisse) below the ano-cutaneous line which has a stratified squamous epithelium, but which is lacking in hairs and glands. As soon as the sebaceous glands begin, they are relatively large.

Herrmann and Defosses ('80) describe another type of gland in the anal mucosa. They assert that at the bottoms of the sinuses limited by the semilunar valves, the epithelium prolongs itself in irregular canals which extend across the internal sphincter, in contact with which they are frequently enlarged, forming widened excavations. From these enlargements branch off several ducts which are quite similar to gland tubes. The tubes traverse the whole of the internal sphincter, and terminate in the connective tissue interposed between this muscle and the longitudinal layer of the muscularis.

Herrmann ('80) goes somewhat more into detail in the description of the glands found in the anal region. Several types are considered. 'Erratic glands' are intestinal glands which are found below the ano-rectal line in the region of the zona columnaris. They are few in number and are found only in a region a few millimeters below the ano-rectal line. 'Isolated goblet cells' are likewise found for a short distance below the ano-rectal line and finally

'special acinous glands' which have already been referred to as the intramuscular glands. In regard to these last structures Herrmann states that he is not certain that they should be classified as glands. He was able to find no trace of secretory epithelium in them. He suggests that the term 'intramuscular sinus' would probably be more appropriate for them. He is nevertheless certain, because of their peculiar disposition that they correspond to the true acinous glands found in this region of the dog. Regarding the structure of the intramuscular glands Herrmann states that those in man are made up of polygonal cells which are similar to those of the epithelium from which they arise. They extend outward through the submucous layer, and, in contact with the internal sphincter muscle, the tube of epithelium presents a swelling which is lined with a cuboidal epithelium composed of one to two layers of cells. The branches arising from this swelling penetrate still further into the muscle and terminate in small caecums in between the inner circular and outer longitudinal layers of muscle. Scattered along the tubes, and particularly about their terminal ramifications, are found masses of lymphoid tissue.

Braun ('01) states that in the transition zone (*zona intermedia*) can be found in some individuals, though not in all, free sebaceous glands. Because he was unable to find any of Herrmann's special acinous glands (intramuscular sinuses) he completely denies their presence. He describes and figures, however, tube-like structures (*röhrenförmige Anhänge*) which arise from the secondary sinuses (*'Sinuositäten'*). These, however, were found in the submucosa and did not pierce the internal sphincter muscle.

Although the glands of the anal region in man have attracted but little attention, those of the lower mammals have been more thoroughly studied. Herrmann uses the term 'anal glands' to denote the two large saccular glands of the dog. Besides these he describes large intramuscular glands, which, unlike those of man, open into the *pars analis recti* below the *zona columnaris*. These are true acinous glands lined with secretory epithelium.

Hebrant ('99) likewise describes under the term 'anal glands' of the dog, the two saccular glands found one on either side of the

anal orifice, and which open by small ducts on the skin just lateral to the anus. He also describes 'sudoriparous' glands which apparently include both Herrmann's intramuscular glands and Zimmermann's 'circum-anal' glands. He also describes the close relation of the lymphoid nodules to the anal mucous membrane of the dog, and remarks upon their close resemblance to the structure of the tonsils. With regard to this he says: "N'y a-t-il pas lieu ainsi d'admettre, de la part des glandes anales par les follicules clos qu'elles renferment, une action analogue à cette jouée par les amygdales à l'autre porte d'entrée du tube digestif?"

Zimmermann ('04) has made a careful and complete study of the glands of the anal region of the dog. He divides the anal mucous membrane into three zones: the zona cutanea, which is composed of internal and external subdivisions, the zona intermedia, and the zona columnaris. All these regions are lined by a stratified squamous epithelium, which takes on more and more the structure of the skin when passing downward from one region into the next. The zona columnaris is characterized by columns, sinuses and valves. The sinuses are not always equally well formed and some 'Sinuositäten' extend in the form of branched canals or crypts into the region of the lymphoid nodules. These structures, he speaks of as the 'anal tonsils.' The zona intermedia is characterized by its smooth epithelium and the ducts of the large 'reservoir glands.' The zona cutanea takes on more and more the structure of the skin when followed downward. The internal portion lies within the anal canal, the external without. This zone contains large sebaceous glands (which according to Zimmermann have incorrectly been called 'circum-anal glands') and sweat glands, both of which open into hair infundibuli in which hair shafts are lacking.

For an account of the anal regions of the domestic animals, the reader is referred to the work of Mladenowitsch ('07) who has studied this portion of the rectum of the horse, ox, sheep, goat, pig, dog, and cat. He finds that the columns of Morgagni, or rectal columns, and the rectal sinuses are constant structures. Blackman ('11) is referred to for an account of the anal glands of the skunk.

Development of the anal glands

Regarding the embryology of the anal glands in man, the work of Herrmann alone is known to the writer. He asserts that the intramuscular glands are present as small epithelial buds in an embryo of 140 mm. In an embryo of 190 mm. they have penetrated the internal sphincter, in which they end in small ampulla-like swellings. At this stage the glands are not provided throughout with lumens, but are in some places solid cords of cells. At 240 mm. they extend through the whole thickness of the internal sphincter muscle and are complete canals. In the region of their terminal swellings lymphoid tissue is already being laid down. At birth they are quite similar to those of the adult.

OBSERVATIONS

Early stages

Under this heading are arbitrarily grouped stages up to 22.8 mm. when the first evidences of the columns of Morgagni are seen. No attempt has been made to work out the early history of the formation of the hind-gut and cloaca, for this has already been thoroughly done by Keibel ('96), whose observations have been recently confirmed by Pohlmann ('11). These observers show clearly the relations of the hind-gut in its earlier stages to the allantois, and of the rectum to the cloaca, and the division of the cloaca into the rectum and the urogenital sinus.

Observations on embryos of 7.5 mm. (H.E.C. 256) and 10 mm. (H.E.C. 1000) have already been reported (Johnson '13). In these stages the rectum has the form of a hollow tube, and in the 10 mm. embryo presents at its lower extremity a spindle-shaped swelling. The cloaca is present but closed off from the outside by the cloacal membrane. These observations are in accordance with those of Keibel and Pohlmann, who state that the cloaca is never open to the outside.

In an embryo of 13.6 mm. (H.E.C. 839) the rectum presents a large spindle-shaped swelling. At the lower end of this swelling the epithelial tube becomes much reduced in size, but just before

the cloaca is reached, it gradually increases in size again. This lower widened portion, which is relatively short, joins the cloaca.

Separation of the rectum and urogenital sinus has taken place in an embryo of 16 mm. (H.E.C. 1322). The spindle-shaped swelling is more pronounced than before, and its epithelium now passes over into that of the outside skin. Just before the epidermis is reached, however, another slight swelling is seen, smaller and more flattened than the swelling above. The lumen of the rectum is continuous above with that of the colon, is larger in the swellings than in between them, and below opens to the exterior by an extremely fine opening. In a second embryo of 16 mm. (H. 133) the epithelial tube has a similar form. The lumen, however, is occluded at its lower end.

In embryos of 17 mm. (H. 58) and 19 mm. (H.E.C. 819) both swellings have increased slightly in size and are more flattened dorso-ventrally. Again the lumen does not open to the outside for at its lower extremity can be seen a mass of cells which fills the lumen.

At 22.8 mm. (H.E.C. 871) the epithelial tube presents a form as seen in figures 7 and 11. The two swellings are again present, the upper of which is much larger and more conspicuous. Both are again slightly flattened dorso-ventrally. A very shallow depression can be perceived running along the ventral surface of the upper swelling thus forming a small infolding of the epithelium into the lumen. This fold, the first of a large number to develop in the walls of the rectum, may be regarded as the earliest appearance of a rectal column.

Throughout all the preceding stages the epithelial tube is made up of a stratified epithelium of two to four layers of cuboidal cells, without distinct cell boundaries, an epithelium quite similar to that found in the whole of the digestive tract in its early stages. In the region of the swellings the epithelial wall becomes correspondingly thicker.

Surrounding the above described epithelial tube of the rectum is seen loose mesenchyma. The circular layer of the muscularis was first seen in an embryo of 16 mm. It extends down only as far as the constriction in between the two swellings, a point to

which Keibel calls attention. A lighter staining zone was seen just outside of the circular layer of muscle, probably the beginning of the nervous layer. At 17 mm. a faint indication of the longitudinal muscle coat is seen. In embryos of 19 mm. and 22.8 mm., both layers of muscle, with the intervening nervous layer, have become more distinct.

Apparently Keibel was the first to describe the two swellings of the rectum referred to above. He shows that they are present and distinct in embryos of 17.5, 18.5, and 25 mm. In the description of his figure 55—a longitudinal section of the rectum from an embryo of 25 mm.—he says:

Wir sehen die Aftergrube, in welches sich die Deckschicht des Ectoderms fortsetzt. Es folgt weiter cranial eine kleine Anschwellung des Darmrohres, die aber mit der Aftergrube noch nicht in offener Verbindung steht. Die Grenze zwischen Ectoderm and Entoderm tritt ziemlich deutlich hervor. Auf die kleine Anschwellung des entodermalen Enddarmrohres folgt cranial eine stark ausgebildete Anschwellung, wie sie ja an den Zeichnungen nach den Modellen und auf der Profilreconstruction klar genug zur Darstellung kommt.

Tourneux ('90) who studied the cloacal region of the sheep, does not refer to these two swellings so distinctly shown by Keibel, and Pohlmann ('11) apparently failed to find them in the human embryo.

Concerning a sagittal section through the rectum of a 22 mm. embryo, Lewis ('12) states that:

Just before the rectum reaches the anal membrane it forms a bulbous enlargement The terminal swelling extends beyond the muscle layers as recorded by Keibel In a 32 mm. specimen the anal membrane has disappeared. Along the dorsal wall there is a slight indication of a terminal bulbous enlargement but it seems clear that it is a transient structure. It is probable that the elongated swelling above it gives rise to the rectal ampulla of the adult.

Lewis accordingly has labelled the elongated upper swelling the 'ampulla recti.' Apparently, however, he disregards the accepted use of 'ampulla recti,' for he applies the term to the bulbous pars analis recti in the figure of a case of atresia ani cum fistula vulvari, copied from Mackenzie. The conjecture that the elongated swelling gives rise to the pars analis recti proves to be correct, but

the term 'ampulla recti' is wholly inapplicable, as will be shown. At the time his article was written, Lewis realized the importance of further study upon this subject, and pointed out to me the desirability of following this swelling throughout the later stages of the embryo.

The upper swelling persists as a definite swelling throughout stages up to birth. It is an embryonic structure quite similar to that found at the junction of the ileum and the colon, which the author ('13) designated by the term 'ampulla coli.' Originally it was likewise intended to use the term 'ampulla recti' for this embryonic structure in the rectum. From its position in the early stages of the embryo one would suppose that it was identical with the ampulla recti of the adult. However, such is not the case. With the growth of the embryo it becomes relatively shorter and shorter and gradually descends lower and lower in the pelvis. It eventually turns out to be a portion of the pars analis recti of the adult, principally the zona columnaris. As this structure has as yet received no definite name, the author proposes the term 'bulbus analis.'

With regard to the lower swelling it can be said that it forms the zona intermedia of the adult pars analis recti, although nothing can be definitely said with respect to its boundaries. It persists as a swelling only throughout the early stages.

The dorsal portion of the cloaca into which the rectum opens has been termed by Tourneux ('90) the 'vestibule anal.' In describing it he says:

L'extrémité inférieure du rectum débouche maintenant dans un vestibule qui se prolonge en avant jusqu'au bouchon cloacal, et qui représente le sommet même de l'anse cloacal unissant primitivement le rectum au bouchon. Nous donnerons à ce vestibule le nom de vestibule anal, et à sa paroi inférieure ou cutanée exclusivement formée de cellules épithéliales, le nom de membrane vestibulaire ou de membrane anale.

When the cloaca becomes divided into dorsal and ventral parts, whether it be by means of the downgrowth of the perineal fold (as described by Tourneux) or by means of a pushing together of the two lateral mesodermic folds (Retterer '90), it is evident that the dorsal half of the cloaca becomes incorporated with the rectum

and forms its lower portion. It is also evident that the lower swelling described in the 16 mm. and 19 mm. embryos, is formed from the old cloaca. This is what is taking place in the above-described 13.6 mm. embryo, where the lower bulb is only partially formed. This conclusion seems justifiable not only because the constriction between the bulbus analis and the cloaca in the early stages is identical with the constriction between the bulbus analis and the lower swelling in the later stages, forming a definite boundary superiorly, but because of the constant and definite relation presented by the anal membrane. This membrane, which is identical with the posterior half of the cloacal membrane, forms the inferior wall of both the anal vestibule in the early stages and of the lower swelling in the later stages. The lower swelling, which develops from a part of the cloaca (Tourneux's 'vestibule anal') and which forms the zona intermedia of the adult pars analis recti, will be spoken of by the term 'bulbus terminalis.'¹

Late stages

The form of the rectum of an embryo at 30 mm. (H.E.C. 913) is shown in figures 8 and 12. It is to be noted that the figures represent the basal surfaces of the epithelium, that is, those surfaces which are covered by mesenchyma. The grooves, therefore, which are seen on the outer surface are to be considered as folds of the mucous membrane turned into the lumen of the rectum. For clearness of description in the remainder of this paper, these folds of mucous membrane into the lumen will always be spoken of as 'in-foldings,' while the furrows in between them will be called 'out-foldings.' The side view shows that the dorso-ventral curvature is slightly greater than at 22.8 mm. The bulbus analis is not well marked off from the intestinal tube above, but below, where it joins the bulbus terminalis there is a great diminution in its dorso-ventral thickness. The bulbus termin-

¹ Since the 'bulbus analis', the 'bulbus terminalis,' and the swelling at the ileo-colic junction are all similar embryonic structures, for the sake of consistency, it would be better perhaps, to designate the latter by the term 'bulbus coli' rather than 'ampulla coli.'

alis itself is only slightly thicker than the constriction. Viewed from in front the upper extremity of the bulbus analis is again indistinctly marked, whilst below its lower boundary is quite sharp. The whole surface of the anterior wall of the bulbus analis is deeply furrowed by a single longitudinal groove. Two other well marked grooves are found, one on either side.

Both the internal circular and the outer longitudinal muscle coats of the rectum are distinct; also the levator ani and external sphincter muscles are apparent. The circular layer of the muscularis of the rectum extends down to the constriction between the swellings of the two zones, but displays no special thickening which characterizes this region in later stages. The outer longitudinal muscle coat is still faintly visible, but its fibers can be seen running beyond the region of the circular coat. They soon become lost in the mesenchyma. The levator ani is seen running an oblique course from above downward and toward the rectum. The external sphincter lies just below its inferior extremity and its fibers are not distinctly marked off from those of the levator ani. Numerous nerve fibers and small blood vessels are seen in the substance of and between the muscle layers.

The epithelium is different in different regions. In the bulbus analis it is 0.036 to 0.045 mm. in thickness and has two to three layers of oval shaped nuclei. Cell boundaries are not distinct. In the region of the anal opening the epithelium is of the same thickness, but the cells of the epithelium are polygonal in shape, and have distinct boundaries. Of the four to five layers of cells, the uppermost are somewhat flattened. In the region of and that adjoining the bulbus terminalis the epithelium is only 0.024 to 0.030 mm. At the lower extremity of the bulbus terminalis is the transition between the two types of epithelia described above. The lumen is pervious throughout, a slight opening being present through the anal membrane.

As regards the degree of development of the rectum, the embryo just described probably represents a retarded stage. This is made evident because in three other embryos of about the same age which were studied—29 mm. (H.E.C. 914), 30 mm. (H. 108), 31 mm. (H. 57)—more advanced conditions were found.

The epithelial tube of the rectum of the embryo of 29 mm. is shown in figures 9 and 13. It will be observed that the three infoldings are well marked, though they are somewhat less developed than in the above-described 30 mm. stage. These infoldings extend themselves throughout the full extent of the bulbus analis and terminate below on the bulbus terminalis. Above they terminate (with the exception of the ventral one) at the upper limit of the swelling. The ventral infolding extends beyond the bulbus analis. It should also be noted that whereas before the bulbus terminalis was flattened dorso-ventrally, it is now flattened laterally. Moreover, a distinct swelling in this region can hardly be said to exist.

The outfoldings of the epithelial tube demand especial attention. It is to be noted that the outfolding forming the left border of the rectum, can be traced into the upper border of the flattened bulbus terminalis. The one on the right terminates on the right side of the bulbus terminalis, while the dorsal passes down directly into the dorsal wall of the same. These conditions suggest that the epithelial tube in its lower part has been twisted through an angle of 90° in a direction which, when looked at from above, is clockwise. Whether any such twisting does take place between the two last described stages is a rather difficult problem to determine. More will be said in regard to this below.

Although the bulbus terminalis shows again as a flattened slit-like canal in embryos of 30 mm. (H. 108) and 31 mm. (H. 57), slight variations are met with in regard to the folds, particularly at the lower end of the zona columnaris. In the 30 mm. stage is found a small additional ventral outfolding arising from about the middle of the ventral infolding. At 31 mm. the left outfolding does not quite reach the upper border of the bulbus terminalis but ends on the side of it near the upper border.

In an embryo 37 mm. (H.E.C. 820) the epithelial tube of the rectum presents a number of long longitudinal folds (figs. 10 and 14). Examination of the model of this rectum shows distinctly that the three outfoldings described in earlier stages are still present, although their identity is somewhat obscured by the presence of additional secondary folds. As in the 29 mm. stage the out-

folding on the left side passes down onto the ventral border of the flattened bullus terminalis, the right terminates on the right side of the bulbus terminalis about half way between the dorsal and ventral borders, while the posterior outfolding forms the dorsal border. Both the right and posterior outfoldings are divided into secondary folds. Just above the bulbus analis five to six irregular folds are seen which undoubtedly represent the pre-villous folds of the ampulla recti. Regarding the outer layers of the rectum, but few changes have taken place. The outer longitudinal coat is more distinct than before and is pierced by numerous small groups of nerve fibers, particularly along its dorsal wall. The external sphincter muscle is seen as a distinct broad band of fibers entirely encircling the lower portion of the rectum. It lies just below the fibers of the levator ani.

As seen in figures 15 and 17, the epithelial tube of the pars analis recti in an embryo of 44.3 mm. (H.E.C. 1611) presents a more advanced condition with regard to folds. Although more secondary folds are present, the primary folds are distinguishable and present similar relations to those of the 37 mm. embryo. Both primary and secondary folds show distinctly in a series of cross sections of the rectum of a 58 mm. embryo. The primary folds are similar in position and relation to those of the former stage. The secondary folds are more numerous, the right primary fold being divided up into six secondary folds, the left into seven, and the dorsal into eight. New secondary folds are seen beginning in between those already present. They develop largely after the manner of villi in the small intestine, by first a thickening of the epithelium and then an invagination of the epithelium along the thickened ridge into the lumen.

A model of the pars analis recti at 65 mm. (H. 55) is shown in figures 16 and 18. With the exception of a few additional secondary folds, the same picture is presented as before. The right primary is made up of five secondary folds, the left of seven, and the dorsal which is much the largest, is composed of about ten secondary folds. Comparison between figures 10, 15, and 16 and figures 14, 17, and 18 will show that the growth of the bulbus

analis has taken place by an outward expansion alone, there being practically no lengthening of this portion of the rectum.

In embryos of 70 mm. and 88 mm. similar relations with regard to folds are seen. The number of secondary folds is only slightly greater than at 65 mm.

Longitudinal sections of the 88 mm. stage show several new points with regard to the epithelium and the outer coats. The transition from the simple columnar epithelium of the ampulla to the stratified squamous epithelium of the skin is not effected by a continuously gradual transition but takes place in three clearly defined stages. The epithelium of the ampulla is simple columnar containing many goblet cells. For the most part the bulbus analis is lined with an epithelium of two to three layers of columnar cells, the basal of which are cuboidal and have deeply staining nuclei. The protoplasm of the cells is distinctly granular. For a short distance below the beginning of this epithelium are found a few scattered goblet cells similar to those in the epithelium above. The transition between this epithelium and that of the ampulla takes place rather gradually at the ano-rectal line. This line, however, does not correspond in position to the constriction between the bulbus analis and rectal ampulla, as will be shown later.

The epithelium of the bulbus terminalis, or zona intermedia as it will hereafter be called, is composed of five to ten layers of polygonal cells, and differs only from that of the skin of the nates in thickness. The transition between this and that of the zona columnaris is again rather gradual. It forms the linea sinuosa analis. The zona intermedia passes directly over into the zona cutanea, the line of division between the two, the ano-cutaneous line, not being developed at this stage. The muscularis mucosae in the region of the ampulla is distinct but its few fibers dwindle out in the region of the bulbus analis.

Practically the same relations are seen in an embryo of 99 mm. The portion of the circular muscle layer which develops into the internal sphincter shows a slight thickening.

In figure 25 is represented one-half of the rectum of a 110 mm. embryo viewed from the inside. The figure was drawn directly

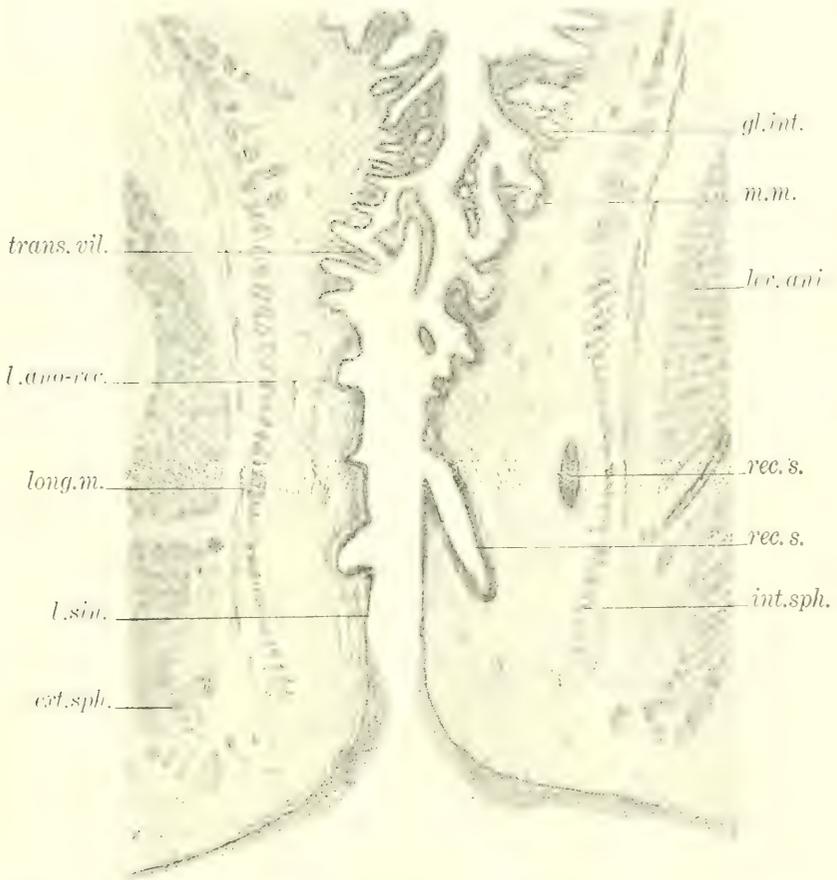


Fig. 1 Longitudinal section through the rectum of a human embryo of 88 mm. $\times 40$ diameters. Between the ano-rectal line and the sinuous line is the zona columnaris lined with stratified columnar epithelium. Below the sinuous line is the zona intermedia. The ano-cutaneous line is not developed at this stage. *ext.sph.*, external sphincter muscle; *gl.int.*, intestinal gland; *int.sph.*, internal sphincter muscle; *l.ano-rec.*, ano-rectal line; *l.sin.*, sinuous line; *lev.ani*, levator ani; *m.m.*, muscularis mucosae; *rec.s.*, rectal sinus; *trans.vil.*, transitory villus of the ampulla recti.

from a dissection with the aid of a magnifier. Rectal columns and sinuses of different sizes show themselves distinctly. At the lower ends of the rectal sinuses can be seen the beginnings of the anal valves. The ano-rectal line cannot be definitely located, but the sinuous anal line is marked by the position of the anal valves. In this specimen the bulbus analis does not seem to be present as a distinct swelling.

Longitudinal series of sections of embryos of 134 and 140 mm. show the addition of numerous secondary folds. Owing, however, to the advantage which transverse sections give to their study, a description of these folds is reserved for the succeeding stage. The outer coats again deserve special attention. The fibers of the muscularis mucosae are now distinctly seen, and extend a short distance below the beginning of the zona columnaris. The circular muscle coat extends slightly below the inferior limit of the same zone and terminates abruptly by becoming slightly thickened. The longitudinal muscle fibers become fewer and fewer when followed down. Some of them extend into the connective tissue septae between the fibers of the external sphincter muscle. Above and to the outside of the external sphincter are seen the fibers of the levator ani.

In a series of transverse sections of the rectum of a well preserved embryo of 135 mm., the pars analis recti presents a condition which, although it contains more secondary folds, is nevertheless greatly similar to that of the above-described embryo of 65 mm. The three primary folds particularly occupy the same position and present the same relations. The bulbus analis again appears as a swelling as is shown in the graphic reconstruction presented in figure 2, A. With respect to its diameter, it is relatively shorter than in the younger stages, although it is actually double the length of the swelling of the 65 mm. embryo. Some idea of its form may be obtained from the cross sections shown in figure 2. In section 600 there are three main folds of the mucous membrane, one ventral and two lateral. A glance at the two following figures will suffice to show that these folds are continued downward on the swelling of the bulbus analis. In section 600 and above it the mucous membrane presents both glands and

villi which are similar to those found in other parts of the large intestine. Intestinal glands are distinctly seen in the figure but the villi are in most places quite low. The epithelium lining this portion of the rectum is simple columnar, composed of cells with a clear protoplasm, many of which are goblet cells. At the basal

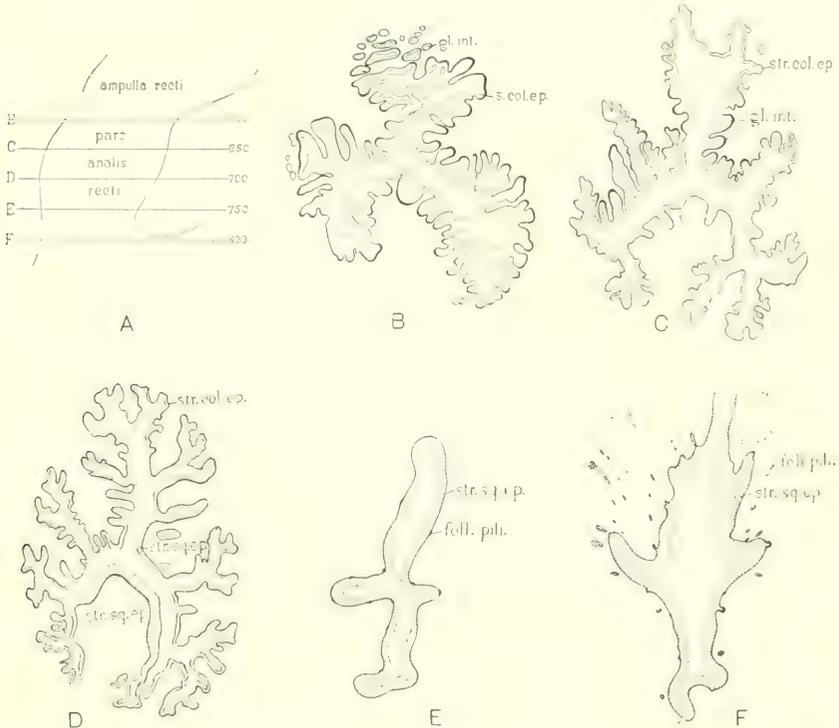


Fig. 2 A. Reconstruction of the lower part of the rectum of an embryo of 135 mm. $\times 5$. B, C, D, E, F. Successive sections taken at the levels as shown in A. $\times 15$. *foll. pili*, hair follicle; *gl. int.*, intestinal gland; *s. col. ep.*, simple columnar epithelium; *str. col. ep.*, stratified columnar epithelium; *str. sq. ep.*, stratified squamous epithelium.

ends of the glands a thin zone of developing muscle fibers, the muscularis mucosae, (0.018 mm. in thickness) is apparent. The muscularis proper is well developed, both layers forming continuous rings around the epithelial tube (together 0.117 mm. thick). Between the two layers of the muscularis are seen numerous nerve

fibers and few nerve cells embedded in the connective tissue. Separated from the outer longitudinal muscle coat by a thin band of connective tissue is seen a third heavy layer of muscle fibers, the levator ani. Already striations can be made out in these fibers. At the level of section 600 the fibers of the levator ani are arranged circularly, but the ring is discontinuous both ventrally and dorsally.

In section 650 the villi and the intestinal glands have for the greater part disappeared, although several can be seen in the immediate region of those labelled in the figure. The epithelium lining these is the same as before. In the remainder of the tube the epithelium is stratified, composed of three to four layers of low columnar cells. The protoplasm of these cells is finely granular and stains more intensely than that of the cells in the glands. The transition between this epithelium and that of the ampulla recti is gradual and forms the ano-rectal line. Since section 650 passes through the bulbus analis, it becomes evident that the histologically-described ano-rectal line lies within this swelling and does not correspond in position to the constriction between the bulbus analis and the ampulla recti. Few goblet cells appear in the stratified epithelium below the ano-rectal line but these become fewer and fewer and finally disappear when followed downward. The remaining outward projections of the epithelium in the figure, namely those which are not glands, are secondary outfoldings.

In section 700 (fig. 2, *D*) glands, villi and intestinal epithelium with its goblet cells, have entirely disappeared. However the stratified columnar epithelium does not prevail throughout for on the summits of the ventral and left primary infoldings, a stratified squamous epithelium has made its appearance. This gradually becomes thicker and more extensive as the anal canal is followed caudally. The transition between this and the stratified columnar epithelium (*linea sinuosa analis*) is, as pointed out by Herrmann, gradual.

The gland-like protuberances seen in the ventral fold of section 700 are merely the downward projections of the ends of folds, similar to those seen all around the tube. A model of some of

these projections is shown in figure 21. In section 700 the muscularis mucosae has almost disappeared but traces of it can be found in the form of a few scattered fibers lying close to the epithelium, particularly at the bases of the folds.

Between sections 700 and 750 are found the lower limits of the secondary folds. These terminate as downward projections in the form of tubules. Those belonging to the left ventral out-folding are represented in figure 21. These tubular structures are lined with a cuboidal epithelium of two to three layers of cells and take on very much the appearance of glands. No evidence that the epithelium is a glandular one, however, was obtainable anywhere. Some of the tubules, about seven in number, turn outward and come in contact with the internal sphincter muscle. Two others pierce the internal sphincter and lie between it and the outer longitudinal layer of muscle, one of which is shown in the figure. It is this type of tubule which Herrmann describes under the term 'intramuscular sinus.'

The lining epithelium of section 750 (fig. 2, *E*) is entirely stratified squamous, composed of seven to ten layers of cells. It presents a few thickenings of the basal layer of cells which mark the beginning of the hair follicles. It would seem from this that this section was near the border line between the zona intermedia and the zona cutanea. The superficial one to three layers of squamous cells take the stain (orange G) more intensely and their nuclei are smaller and more deeply staining than those of the remaining layers, marking the beginning of a stratum corneum.

In the section 800 (fig. 2, *F*) the zona cutanea proper is reached. Here the epithelium is slightly thicker than above, but its chief characteristic is the presence of numerous hair follicles. The irregularities of the epithelium are skin folds which extend radially from the anal opening. Testut ('95) refers to these under the term "plis radiés de l'anus" and Symington ('12) as the "anal skin folds."

The longitudinal section of the rectum of an embryo of 187 mm. represented in figure 3 shows well the topographical relations of the various parts and structures of this region. The ampulla recti is lined with the simple columnar epithelium which for the

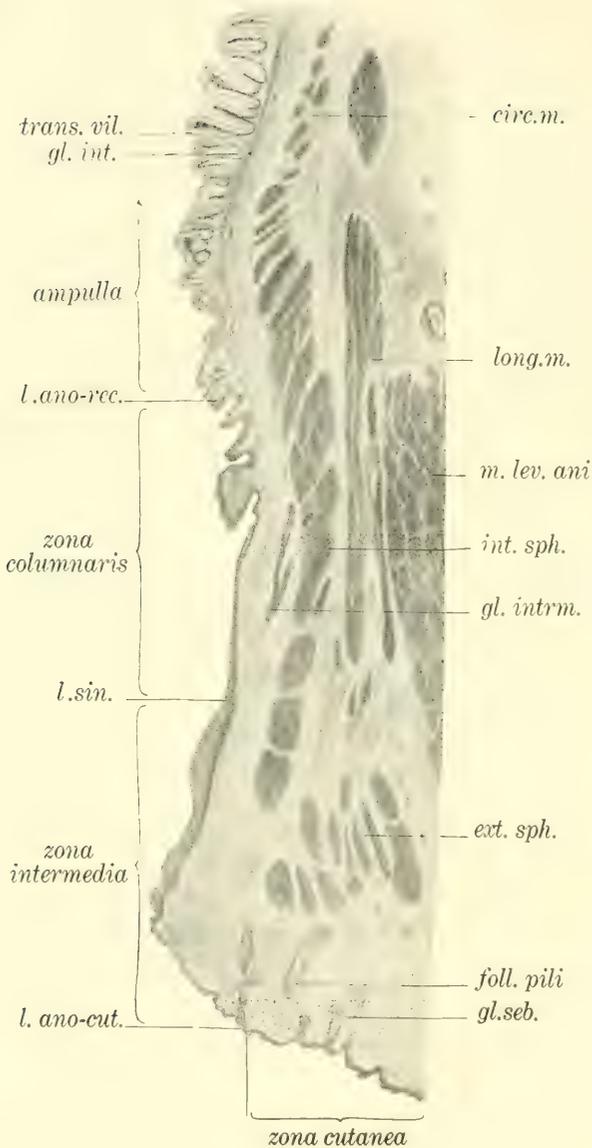


Fig. 3 Longitudinal section through the pars analis recti. From a human embryo of 187 mm. *ampulla*, ampulla recti; *circ. m.*, and *long. m.*, circular and longitudinal layers of the muscularis; *foll. pili*, hair follicle; *gl. int.*, intestinal gland; *gl. intrm.*, intramuscular gland; *gl. seb.*, sebaceous gland; *l. ano-cut.*, ano-cutaneous line; *l. ano-rec.*, ano-rectal line; *l. sin.*, sinuous line; *m. lev. ani.*, levator ani muscle; *ext. sph.*, external sphincter muscle of the anus; *int. sph.*, internal sphincter muscle of the anus; *trans. vil.*, transitory villus of the ampulla.

most part is made up of large goblet cells. The villi are quite low but everywhere in evidence. In passing aborally into the pars analis recti the glands become further spread apart, the goblet cells become fewer and fewer and the epithelium gradually changes over into a stratified columnar type. Although the histological ano-rectal line is not so sharply marked in this section as in many others, nevertheless, it is clear that few goblet cells are present in the upper part of the zona columnaris.

The bulbus analis presents numerous folds as in the former embryos but these are not seen in the figure, owing to the fact that the section passed directly through one of the large infoldings of the epithelium. Extending into the connective tissue subjacent to the epithelium are to be found here and there throughout the series, long tubular projections of the epithelium. In some cases they extend only as far as the internal sphincter while in others they pierce this muscular layer. One of them is shown in figure 3, *gl. intrm.* It extends in an outward and oral direction and divides into three or four branches, only one of which penetrates the muscularis. These glands are lined with a cuboidal epithelium of two to three layers of cells. The inner layer consists of cubical cells with distinct boundaries, a granular protoplasm and rounded nuclei. The outer cells are usually small, they have no distinct boundaries, and their nuclei are smaller and more deeply staining. For the most part the intramuscular tubules of this embryo project upward (orally) but not always, for few were seen extending outwards and downwards. The transition from the stratified columnar epithelium of the zona columnaris to the stratified squamous epithelium of the zona intermedia takes place gradually, forming the sinuous anal line.

The zona intermedia presents a thickened region of the epithelium. The stratified squamous epithelium is from ten to fourteen layers of cells thick in the upper part of this zone, but in the lower part it gradually becomes thinner. The basal two layers of cells are granular, and have large round and oval nuclei. The middle layers of cells are vesicular and polygonal in shape. They are made up of a faintly staining, finely granular protoplasm, and contain small but deeply staining irregular nuclei. The top two

or three layers of cells are flattened and in the lower part of the zone, appear cornified.

The ano-cutaneous line is marked by the beginning of the first hair follicles. Beyond this line is the zona cutanea. The hair follicles are accompanied by beginning sebaceous glands. Still further out are found the beginnings of a few scattered sweat glands which undoubtedly represent the "circum-anal glands" of Gay. A few dermal papillae are also present in this zone. The marked difference between this stage and the stages of 135 mm. and 140 mm. in regard to the disposition of hair follicles can only be explained as individual variations.

The muscularis mucosa (fig. 3) is distinctly seen in the region of the Lieberkühn glands and a few fibers can be traced down into the zona columnaris. The thickening of the circular muscle coat to form the internal sphincter begins in the lower portion of the ampulla and extends down into the region of the zona intermedia a distance of 3.5 to 4.5 mm.

The external sphincter is placed below and to the outside of the internal sphincter and is again separated into numerous bundles of fibers by connective tissue septae. The fibers of the longitudinal muscle layer seem to terminate just before reaching the external sphincter by ending in the connective tissue septae. Above and to the outside of the external sphincter is the levator ani.

A model of the pars analis recti of an embryo of 240 mm. is represented in figures 19 and 20. The ampulla is shown above as cut off at a point where it has about reached its greatest width. It is separated from the bulbus analis by a constricted zone which, as determined from the sections of this series, lies a little above the ano-rectal line. This portion of the rectum (ampulla) presents only intestinal glands, villi having entirely disappeared. Owing, however, to the difficulty met with in modeling the glands at the magnification used (24 diameters) no attempt has been made to represent them. All the folds of the pars analis recti have been modelled. The primary folds can be best seen in cross sections of this region but they are distinguishable in the model. The posterior, right, and left primary outfoldings are present as in the youngest stages, and the left, as seen in the figures, passes

down onto the anterior border of the flattened zona intermedia. The posterior primary fold passes into the posterior border of the same, while the right fold terminates on the side of the zona intermedia. The secondary folds are more numerous than in the above-described stages. Of these there are about twelve on the left primary fold, twenty-two on the right, and about twenty-six on the left, about sixty in all.

Extending outward from this region are to be seen the intramuscular glands. These are more highly developed in the lower portions than above. Some few which did not lend themselves easily to modeling are not shown in the figures. The intramuscular glands extend out from the stratified columnar epithelium of the zona columnaris. Some of them are solid cords of cells throughout, others have distinct lumens, while still others have lumens only in certain portions. Those with lumens are lined by an epithelium of two to three layers of cells, the uppermost of which are in many places columnar and suggestive of secretory cells.

The zona intermedia, owing to distention caused by the presence of meconium in this region, is not so flattened as in the preceding stages. The larger folds of the zona columnaris are prolonged down onto this region and give it an irregular appearance. Several of these folds are directly continuous with the skin folds of the zona cutanea. The zona intermedia is lined with a stratified squamous epithelium of from six to fifteen layers of cells, the uppermost of which show distinct cornification. The epithelium is thickest between the infoldings (0.07 mm.) and thinnest on their tops (0.32 mm.). With the exception of one sebaceous gland (without a hair shaft) which is present high up on the right side of the zona intermedia, no other glands or follicles are present in this region. A considerable distance further down hair follicles and sebaceous glands appear and mark the beginning of the zona cutanea. The zona cutanea is lined with a stratified squamous epithelium of 0.03 to 0.30 mm. thick and is composed of five to ten layers of cells. It is distinctly cornified and shows numerous papillae, hair follicles, and sebaceous glands.

In another embryo of about the same age, (length 245 mm.) a few noteworthy differences were observed. The ampulla and pars analis were both distended with meconium. The series of sections includes the whole of the zona cutanea, the zona intermedia, but only a portion of the zona columnaris. This latter,

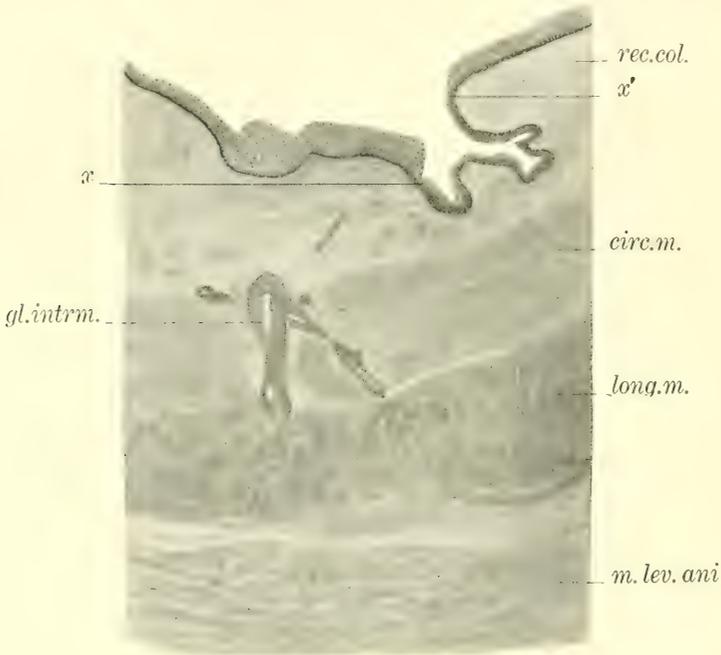


Fig. 4 Portion of a cross section of the pars analis recti of a human embryo of 245 mm. $\times 30$. Between x and x' is a rectal sinus, lined with a stratified columnar epithelium. It belongs to the zona columnaris. The remainder of the surface epithelium is stratified squamous, and belongs to the zona intermedia. *circ.m.*, and *long.m.*, circular and longitudinal layers of the muscularis; *gl.intrm.*, intramuscular gland; *m.lev.ani*, levator ani muscle; *rec.col.*, rectal column; x and x' , sinuous line.

while its lumen is relatively large, shows distinct folds of its mucous membrane quite similar to those of the other stages described.

The epithelium lining the tube of the uppermost section of the series is for the most part stratified columnar, although it is stratified squamous on the tops of the larger infoldings and again in

few of the outfoldings between them. Unlike in the preceding stages the six distinct intramuscular glands found in this series arise from the upper portion of the stratified squamous epithelium, that is, from the *zona intermedia*. All are distinctly tubular and have distinct lumens throughout. For a short distance they are lined with a stratified squamous epithelium but this soon passes over into a stratified columnar which in the branches becomes cuboidal. In all these glands evidences of a secretory epithelium are apparent. One gland, of which a model is shown in figure 23, extends more than half way through the longitudinal muscle coat, giving off at various levels several branches. The primary duct of the gland courses in an outward and downward direction until it reaches the internal sphincter muscle. It then assumes a horizontal direction and in the substance of the internal sphincter, presents a flattened ampulla-like dilation. From this ampulla branches arise which extend on through the internal sphincter and terminate in the connective tissue between the muscle layers. The gland labelled *B* in the same figure represents a simple tubular type which extends only to the internal sphincter but does not pierce it. Another simple gland is shown in figure 22. It has a somewhat ampullated ending at the outer border of the internal sphincter. Several small knob-like protuberances mark what seem to be acini. They are lined with a cuboidal epithelium which is distinctly of a secretory character.

The *zona intermedia* is lined with a thick stratified squamous epithelium which is cornified. This is thrown into a few large folds which are directly continuous with the larger folds of the *zona columnaris* above and with the anal skin folds below. The *zona cutanea* presents no special features.

The external sphincter muscle is of interest in that it gives off vertical columns of striated muscle which extend up into some of the larger folds for varying distances. Likewise small bundles of smooth muscle fibers pass horizontally or obliquely toward the epithelium and also turn upward into the large mucosal folds. Both types of muscle fibers can be traced up into the *zona columnaris*, where they terminate. No evidence of such fibers are apparent in this region of the previously-described embryos.

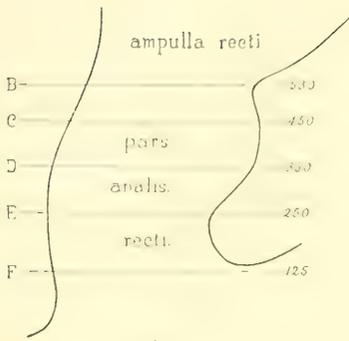
The rectum at birth has been studied from five different specimens. Of these series 10.8 and 10.23 were cut sagittally, series 10.20, 10.21, and 10.22 were cut in cross sections.

The form of the rectum is best seen in the latter two series. Neither of these present any notable differences in form from that of the 240 mm. embryo described above. As shown in a model of series 10.22 (not figured) the primary and secondary folds correspond very closely with those shown in figures 10 and 20. The lumen and size of the tube, however, is somewhat smaller, owing undoubtedly to less distention. Series 10.20 presents what appears to be a flattened and distorted condition of the whole pars analis recti. However, as the preservation of this specimen is poor, a description of it will be omitted.

The form of series 10.21 is shown in figure 5. In *B* is represented a section through the lower portion of the ampulla showing true intestinal glands and no villi. In *C* is seen a section through the zona columnaris. The three primary and numerous secondary folds are quite distinct. Section *D* is through the upper part of the zona intermedia. Several intramuscular glands, which arise from the zona columnaris higher up, are seen just outside the epithelial tube. In *E* is shown a section taken through the lower portion of the zona intermedia. The sharp irregular folds have given way to larger and more rounded ones. The three primary folds terminate in this zone in a manner as described for the preceding stages. Section *F* shows numerous sebaceous glands and hair follicles, and is therefore through the zona cutanea.

The relations of the different zones is well shown in sagittal series 10.8. The zona columnaris, like in the preceding stages, is lined with a stratified columnar epithelium. The sinuous anal line is distinctly marked by a sharp transition from the stratified columnar epithelium to the stratified squamous epithelium. The

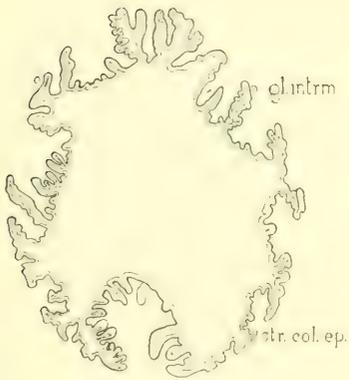
Fig. 5 *A*, reconstruction of the lower part of the rectum of a full term fetus. $\times 4$. *B*, *C*, *D*, *E*, *F*. Successive sections of the rectum taken at the levels as shown in *A*. $\times 8$. *foll.pili.*, hair follicle; *gl.int.*, intestinal gland; *gl.intrm.*, intramuscular gland; *gl.seb.*, sebaceous gland; *s.col.ep.*, simple columnar epithelium; *str.col.ep.*, stratified columnar epithelium; *str.sq.ep.*, stratified squamous epithelium.



A



B



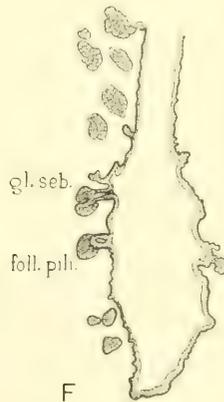
C



D



E



F

Fig. 5

epithelium of the zona intermedia is much thicker than that of the zona columnaris and shows only a slight amount of cornification. This, however, increases when followed downward so that at the ano-cutaneous line the stratum corneum is as thick as that of the zona cutanea. The description of the "zona cutanée lisse" of Robin and Cadiat is in agreement with what is found in the zona intermedia at birth. It is a smooth zone, contains no hair follicles, sebaceous or sweat glands and only a few dermal papillae. These latter are found only in the lower part of the zone. The ano-cutaneous line is arbitrarily placed at the point where the hair follicles and sebaceous glands begin to appear. A short distance beyond this point are found numerous sweat glands, which represent the circumanal glands of Gay.

Intramuscular glands were found in series 10.20, 10.21 and 10.22. In all three series they arise from the bottoms of the outfoldings of the zona columnaris. In structure they are similar to those seen at 240 mm. Although evidences of secretory cells are rare in these glands at birth, few were seen in different places.

No intramuscular glands were found in series 10.8 and 10.23 but as these series only contained a few sections each, it is not improbable that the glands were present in the specimens but that none happened to be cut through in these sections.

No marked differences were observed in the outer coats of the rectum. In 10.22 distinct but small bundles of striated muscle fibers were seen extending up from the external sphincter muscle into the folds of the zona columnaris. In some instances these fibers are closely grouped together; in others, they are more widely separated. About six groups in all were present, containing from ten to twenty-five fibers each. In none of the other series were similar fibers found.

In all the rectums at birth the muscularis mucosae is distinct in the region of the ampulla but cannot be distinctly traced down as muscle columns in the large mucosal folds. As in the preceding stages, a few fibers of this muscle layer can be traced below the ano-rectal line into the zona columnaris. These lie quite close to the epithelium and in the lower part of the zona columnaris become lost in the connective tissue.

Plicae transversales recti

The development of the plicae transversales recti has been described by Baur ('60). The few observations given below are essentially in accordance with those of Baur. Apparently these folds develop in a manner quite similar to the plicae circulares of the small intestine and the plicae semilunares of the large intestine, that is, by a gradual infolding of the mucous membrane.

In an embryo of 70 mm. a careful macroscopic examination of the rectal wall showed no evidence of transverse folds. At 99 mm., however, two small folds are perceptible in the lower portion of the ampulla. At 140 mm. the transverse folds, which are quite distinct, are arranged somewhat obliquely around the wall of the ampulla recti. Three such folds are present, placed at distances of 10 to 13 mm., 15 to 18 mm., and 20 to 22 mm. above the anal opening.

In an embryo of 187 mm. two small folds are present on the right side at distances of 20 mm. and 25 mm. above the anus, while a third larger fold is present on the left side, 30 mm. above the anal opening.

Out of four fetuses at birth the plicae transversales are present in three. In the first case the lowest fold is on the left side 30 mm. above the anal opening. Above this fold are two others both on the right side at distances 40 mm. and 50 mm. above the anus. As seen in figure 24, these folds extend around onto the left side for a short distance. In the second fetus at birth, only two folds are present, the lower being on the left side, while the upper is on the right. These lie at distances of 35 mm. and 45 mm. above the anus respectively. The third fetus likewise has two folds, but the lower is on the right side and the higher on the left, at distances of 30 mm. and 40 mm. above the anus. In the fourth fetus the plicae are entirely absent. The ampulla, however, is very much distended with meconium, which probably in part accounted for their absence.

These observations are not entirely in accordance with those of Otis ('87) who found that when two folds were present the lowest was on the right side and the upper on the left, while when three folds were present, the lowest was on the left side.

With regard to the direction of the folds it may be said that in practically no case did the folds run directly transverse to the long axis of the rectum. Usually they were placed somewhat obliquely. However, in no instance did they have a distinct spiral arrangement around the wall of the rectum, after the manner described by Vance.

The relation of the developing rectum to the vertebral column

Along with the development and descent of the various structures and organs of the pelvis, there take place corresponding changes in the position of the rectum. These changes are particularly noticeable in case of the pars analis recti which, in its early stages, lies at a higher level and is relatively greater in its extent than in the later stages. Whereas in the adult the first portion of the rectum begins at the level of the third sacral vertebra (by definition) and lies along the concavity of the remainder of the sacrum and coccyx, in very early stages this portion of the rectum is found above the third sacral vertebra. Likewise the pars analis recti which in the adult is an inch or more in front of and below the tip of the coccyx, early is found occupying a position extending from the third sacral vertebra to the tip of the coccyx. The determination of such relations has only been made possible through the existence of the bulbus analis, which as has been shown, is present as a swelling throughout stages up to birth, and which forms in a large part the zona columnaris.

In an embryo of 16 mm. (H. 133) the spindle-shaped bulbus analis lies along the concavity of the sacrum. Its upper limit lies on a level with the third or fourth sacral vertebra. The anus is found in the neighborhood of the second or third coccygeal vertebra. Such levels, however, can only be approximately determined. This is because the epithelial tube of the rectum does not lie directly against the bodies of the vertebra, and because of the great curvature of the body in this region. The long axis of the embryo in this region, therefore, is a much curved line.

In figure 6, A are seen the relations which exist in an embryo of 28.8 mm. (H.E.C. 1598). The upper portion of the bulbus

analis again lies at about the level of the third or fourth sacral vertebra, while the anal opening lies at about the same level as the tip of the coccyx.

In embryos of 31 mm. (H. 57) and 35 mm. (H. 51) the bulbus analis reaches only as high as the fifth sacral vertebra, while the anal opening presents the same relations as in the preceding stage.

In an embryo of 50 mm. (H. 115, fig. 6, *B*) the bulbus analis is relatively much shorter. Its upper limit now lies on a level with the second or third coccygeal vertebra, while the anal opening is found on about the same level as the tip of the coccyx. At

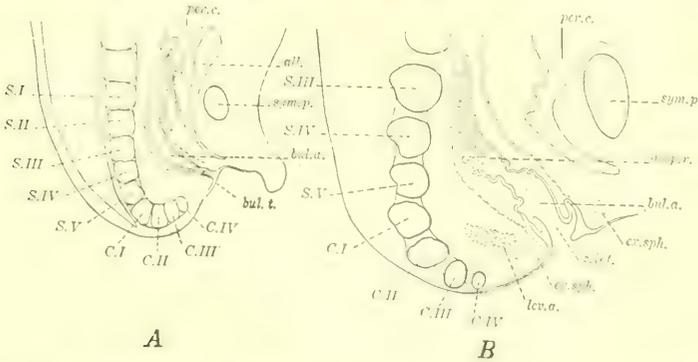


Fig. 6 Sagittal section through the anal regions of human embryos. *A*, 28.8 mm., (H.E.C. 1598) and *B*, 50 mm. (H. 115). $\times 6$. *all.*, allantois; *amp.r.*, ampulla recti; *bul.a.*, bulbus analis; *bul.t.*, bulbus terminalis; *C.I* to *IV*, coccygeal vertebrae; *ex.sph.*, external sphincter muscle; *lev.a.*, levator ani muscle; *per.c.*, peritoneal cavity; *S.I.* to *V.*, sacral vertebrae; *sym.p.*, symphysis pubis; *z.int.*, zona intermedia.

140 mm. the curvature of the sacrum and coccyx is less than before, and the bulbus analis lies entirely below the tip of the coccyx. At birth practically the adult relations are met with (fig. 24).

The limit between the entoderm and ectoderm

Retterer ('90) in a study of the early stages of embryos of the sheep, pig, and rabbit, concluded that the mucous membrane of the anal region is derived directly from the ectoderm and the underlying connective tissue. Herrmann ('80) likewise believed that the whole of the pars analis recti is derived from the ecto-

derm, the line of separation between the ectoderm and entoderm being represented by the ano-rectal line. His reason for maintaining this view was because of the change in the character of the epithelium at this point. The few isolated intestinal glands ('glandes erratiques') and goblet cells he regards as fragments of entodermic epithelium which have descended into the ectoderm of the anal region.

After the breaking down of the anal membrane, there is no apparent line of demarcation between the entoderm and the ectoderm. However, when the cloacal or anal membranes are present, the junction of the two germ layers is definitely marked. This junction lies just below the *bulbus terminalis*. Since both the *bulbus analis* and the *bulbus terminalis* lie above the anal membrane they are composed of entoderm and it is evident, therefore, that the derivatives of these, the *zona columnaris* and the *zona intermedia* of the *pars analis recti*, are of entodermal origin. The exact line of demarcation in the adult cannot be definitely located, but it is undoubtedly somewhere in the neighborhood of the *linea ano-cutanea*.

DISCUSSION AND SUMMARY

A study of a few young stages of the human embryo confirmed the results of Keibel and Pohlman in that the cloaca in the human embryo is never open to the outside, and that division of the cloaca into urogenital sinus and rectum takes place in embryos of about 16 mm. in length. After separation has taken place, the posterior portion of the cloacal membrane persists as the anal membrane and occludes the rectum at its lower extremity. It is present for some little time and exists in the form of a few irregular cells similar to those of the wall of the anus. This was last observed in an embryo of 22.8 mm. An exception to this is found in the 16 mm. embryo, in which the anal canal is already open to the outside. Keibel ('08), however, shows that ordinarily the anus is closed in embryos as old as 26 mm., while Broman ('11) states that its lumen becomes patent in embryos of about 33 mm.

In embryos of 7 and 10 mm. the rectal tube presents a spindle-shaped swelling. It is apparent as a swelling throughout stages

up to birth, although its form changes greatly. At first it is elongated. It increases in size proportionally up to the 37 mm. stage. Little or no increase in length occurs throughout stages 42, 44.3 and 65 mm., although the increase in its cross diameter is considerable. From this stage on there is an increase in the length of the swelling, but the direction of greatest growth is transversely. Whereas in the earlier stages its length is several times its diameter, at birth the diameter slightly exceeds its length.

In embryos of 13.6 and 16 mm. there appears below the above-described spindle-shaped swelling a second swelling. This is much shorter, and not so well marked as the upper. It exists as a swelling only for a short period of embryonic life. In an embryo of 29 mm. the swelling has largely disappeared, while in embryos of 30 mm. and over it is represented as a narrow slit-like cavity.

Keibel and Lewis have described these swellings of the rectal tube in corresponding stages of development. The upper of the two swellings, which I have termed the 'bulbus analis,' occupies a position in the embryo which corresponds quite closely to that occupied by the ampulla recti of the adult. However, as has been shown, it does not form the adult rectal ampulla. The walls of the embryonic swelling become invaginated and form numerous longitudinal folds and ridges. The study of these folds throughout successive stages of development show that they are the rectal columns. As these are found principally within the zona columnaris of the pars analis recti, it follows that the spindle-shaped swelling of the embryo becomes in large part the zona columnaris of the adult. The upper portion of the bulbus analis, however, lies above the ano-rectal line, and from the standpoint of histology, belongs to the ampulla recti. The lower swelling, which I have termed the 'bulbus terminalis,' develops from the dorsal portion of the cloaca which Tourneux has called the 'vestibule anal.' It is limited above by the constriction between it and the bulbus analis, and below by the anal membrane. It forms the lower portion of the pars analis recti, namely the zona intermedia. It cannot be definitely said, however, in

regard to either of the two swellings that their boundaries correspond absolutely to the boundaries of the two above-named zones.

The first evidence of the development of folds in the bulbus analis was found in an embryo of 22.8 mm. At 30 mm. three distinct folds are present, the outfoldings of the epithelium occupying positions posteriorly, and on the right and left sides. These three primary folds are present throughout stages up to birth. Whether they are present in post-natal rectums has not been determined. They extend throughout the whole length of the bulbus analis. Below they terminate by ending on the zona intermedia. This, in stages up to about 30 mm., is flattened ventro-dorsally. The right and left primary folds become directly continuous with the right and left borders of the bulbus terminalis respectively. In stages of 31 mm. and over, the bulbus terminalis or zona intermedia is flattened laterally. This band-like portion of the rectum, therefore, either undergoes a rapid change in its form or in its position. Just what happens at this stage was not clearly brought out by the embryos examined. From the position occupied by the left fold, in the later stages, a twisting to the right through an angle of 90 degrees seems to have taken place. However, from the arrangement of the other two primary folds and the tissues in the immediate neighborhood of epithelial tube, it is not probable that any such twisting has gone on.

In all the later stages of development the constancy with which the primary folds are found is very striking. Moreover, these folds terminate at their lower extremities in a similar manner in almost all stages examined. The outfolding on the left side passes up onto the ventral border of the laterally flattened zona intermedia; the posterior fold extends directly into the dorsal border of the same; while the right fold extends as far as the zona intermedia and terminates on its right side, about half way between its ventral and dorsal borders.

In an embryo of 37 mm. secondary folds begin to appear on the bulbus analis. These are longitudinal folds similar to, but smaller than, the primary folds. They develop rapidly and soon

obscure the primary folds. At 54 mm. the secondary folds are quite numerous. Most of them are formed in an embryo of 135 mm., but an embryo of 240 mm. shows some increase in their numbers. Apparently none are formed after this stage.

The significance of these longitudinal folds of the *bulbus analis* cannot be questioned. The infoldings of the mucosa form the *columnae rectales* (columns of Morgagni). When viewed from the inside (fig. 25) the primary folds are not distinguishable and only the larger columns are apparent. Numerous small secondary folds lie at the bottom of the outfoldings or sinuses. The number of such vertical folds or columns is large, there being as many as 60 in a 240 mm. embryo. The smaller of these folds, so far as general structure is concerned, do not differ from the larger columns and should, therefore, be considered as the equivalents of the usually-described rectal column.

The rectal sinuses (*sinus rectales*) of the adult, as they are usually described, are found at the lower extremities of the inter-columnar clefts. An examination of models shows that such pockets are present as the lower portions of the outfoldings, which in places extend down below the general level of the mouth or opening of the cleft.

The anal valves are found at the lower limits of the folds. In the embryo these do not have so much the appearance of the aortic valves as is usually figured for the adult, but are narrower and thicker. It seems reasonable to the author that distention must play an important part in the further formation of the valves and sinuses. The great variation in number, size, and shape in the same and in different individuals, and the extent to which the *pars analis* later becomes distended, would seem to be evidence in support of this view.

The histology of the rectum throughout the different periods of development may next be briefly reviewed and summarized. The first part of the rectum including the ampulla is similar in its development to the colon as has been previously described (Johnson '13).

In the earlier stages of development the *pars analis recti* is lined by an epithelium composed of two to three layers of low

columnar cells. This epithelium is similar to that found in other parts of the digestive tube in its early stages. Differentiation was first noted in an embryo of 55 mm. But few changes take place from this time until birth. The following description, although taken largely from an embryo of 240 mm., will hold good for any of the later stages.

The pars analis recti is the region in which takes place the transition between the mucosa of the digestive tube and the skin. This transition does not take place gradually but in three separate steps, thus forming three distinct superimposed zones, the zona columnaris, the zona intermedia, and the zona cutanea. The last of these zones, however, does not belong to the pars analis, properly speaking, but to the outer skin.

The zona columnaris is the region of the rectal columns, but these, however, are not always confined to this zone alone. They may extend up into the first part of the rectum for a short distance, and they may also be continuous with the anal skin folds which extend inward and upward from below. The zona columnaris is lined by a stratified columnar epithelium of from two to three layers of cells. The transition from the simple columnar to the stratified columnar epithelium takes place rather gradually. It forms the linea ano-rectale. In the upper part of the zone are to be found usually a few intestinal glands and goblet cells.

In the lower part of the zona columnaris, arising from the rectal sinuses, are found tubular gland-like structures. These may be of a simple tubular form lying completely within the submucosa, or they may be branched tubules which pierce the underlying muscle layers. The latter type are known as the intramuscular glands. There are seldom more than six or eight of these in any one rectum. The main ducts of the glands extend outward, and usually downwards, and penetrate the internal sphincter muscle. Here an ampulla-like swelling is usually met with. Extending beyond this ampulla are several tubular branches which continue through the internal sphincter and end blindly in the intramuscular connective tissue. Occasionally a tubule is seen piercing the longitudinal muscle layer. Around the terminations of the tubules which sometimes take on the appearance

of acini, is to be found a small amount of lymphoid tissue. The epithelium lining the glands is made up of several layers of polygonal cells in the main ducts, but in the ampullae and branches it is composed of one to two layers of cuboidal cells. It is claimed that evidences of secretory cells are wanting in the adult, but in the fetus and at birth they are present.

In his summary, Braun tabulates the epithelia of the zona columnaris as follows:

(a) Einfaches Cylinderepithel findet sich in dem proximalen Teil der Columnen; in dem proximalen Teil der Sinus; in allen Lieberkühnschen Drüsen; am unteren, blinden Ende der röhrenförmigen Anhänge der Ausbuchtungen der Sinus.

(b) Geschichtetes Cylinderepithel findet sich in dem distalen Abschnitte der Sinus; auf den die Sinus begrenzenden Flächen der Columnen; in den sekundären Ausbuchtungen der Sinus; in den Ausführungsgängen der röhrenförmigen Anhänge der Ausbuchtungen.

(c) Geschichtetes Plattenepithel (polyedrisches Epithel) findet sich auf der Höhe der Columnen in ihrem distalen Abschnitt.

The results of my own work regarding the epithelium of the zona columnaris are in accordance with the above tabulation, but I must also add that:

(d) Stratified cuboidal epithelium is found in the larger ducts of the intramuscular glands.

(e) Simple cuboidal epithelium is found in the branches of the intramuscular glands.

The zone intermedia is lined by a stratified squamous epithelium. This is composed of several layers of polygonal cells forming a thicker layer than the epidermis of the skin. Dermal papillae are present, but hairs and sweat glands are absent. In the lower part of this zone may be found a few isolated sebaceous glands without hairs, and a slight amount of cornification of the epithelium. The transition between the epithelium of this zone and that of the zona columnaris is rather abrupt. It lies at the level of the anal valves, but in between the valves it extends upward on the summits of the rectal columns. It thus forms a zig-zag line, which is known as the *linea sinuosa analis* (ano-cutaneous line of Herrmann). At its lower limit the zona intermedia gradually goes over into the skin. The transition here forms the true

linea ano-cutanea, but it is not well marked. It has been defined as the place where the first hair follicles appear. The skin in the immediate neighborhood of the anus is known as the zona cutanea. Sweat glands, the circum-anal glands of Gay, are found only in this zone.

The muscularis mucosae in the first part of the rectum is similar to that of the colon. It is said to extend downward in the form of muscle columns into the rectal columns. In the embryos I have examined nothing in the way of distinct muscle columns were to be found. The muscularis mucosae extends into the zona columnaris as a thin sheet of fibers lying quite close to the epithelium. The fibers forming this sheet soon disappear, however. In two stages, 240 mm. and at birth, small bundles of voluntary striated muscle fibers are present in the larger rectal columns. These are composed of a number of fibers which arise from the external sphincter muscle turned obliquely toward the submucosa and then upward into the columns. A few fibers of the internal sphincter join some of these columns in the 240 mm. embryo.

The submucosa, while at first composed of mesenchyma, later becomes fibrous. It contains numerous nerve fibers and blood vessels, and Pacinian corpuscles as described by Pilliet ('92).

The muscularis of the rectum is the downward continuation of that of the colon. Both layers form, however, continuous sheaths around the rectum. The circular was first seen in an embryo of 16 mm., while the longitudinal appeared at 17 mm. The circular layer presents, in the region of the pars analis recti, a thickening, the sphincter ani internus. This does not become apparent until relatively late embryonic life (99 mm. embryo). The longitudinal muscle coat terminates below, after the manner described by Béraud ('58) and Roux ('81), by a spreading out of its fibers. These end in small connective tissue fibers, which, continuing downward, spread out fan-shaped, and as septae, divide up the muscle bundles of the external sphincter.

The external sphincter appears quite early. It was first definitely made out in an embryo of 22.8 mm. According to Otis ('05) the external sphincter arises from the mesenchyma within

the anal papillae (slight elevations on the outside of the anus). He detects the anlagen of the fibers of this muscle in an embryo of 12.5 mm.

Mention should be made regarding the extent and relations of the various muscles to the different parts of the *pars analis recti*. In the early stages, both the circular and longitudinal layers of the muscularis terminate at the constriction between the *bulbus analis* and the *bulbus terminalis*. In the later embryonic stages the circular muscle extends well into the region of the *zona intermedia*, while the longitudinal layer extending not quite so far down, terminates just above the external sphincter muscle. The external sphincter muscle surrounds the middle part of the *zona intermedia*.

The development of the *plicae transversales recti* may be summarized in few words. They apparently develop after the manner of the folds of the large and small intestines, that is, by a gradual infolding of the mucous membrane. They were first seen in an embryo of 99 mm. From this stage on they become more pronounced. Gradually they spread further and further apart with the growth of the rectum as a whole.

A study of the developmental topography of the *pars analis recti* shows that it forms a relatively much greater portion of the digestive tube in the embryo than in the adult. Its growth in length, therefore, is proportionally less than its growth in diameter. With this relative shortening, the *pars analis recti* gradually descends in the pelvis. Whereas in the early stages the whole of the *pars analis recti* lies between the third sacral vertebra and the tip of the coccyx, at birth it lies completely below the tip of the coccyx.

In the early stages of the embryo the meeting point of the entoderm and the ectoderm lies at the lower end of the *bulbus terminalis* at the anal membrane. Since it has been shown that the *bulbus terminalis* develops into the *zona intermedia*, it follows that at birth and in the adult the limit between the two germ layers must lie somewhere near the lower boundary of this zone, that is, near the ano-cutaneous line.

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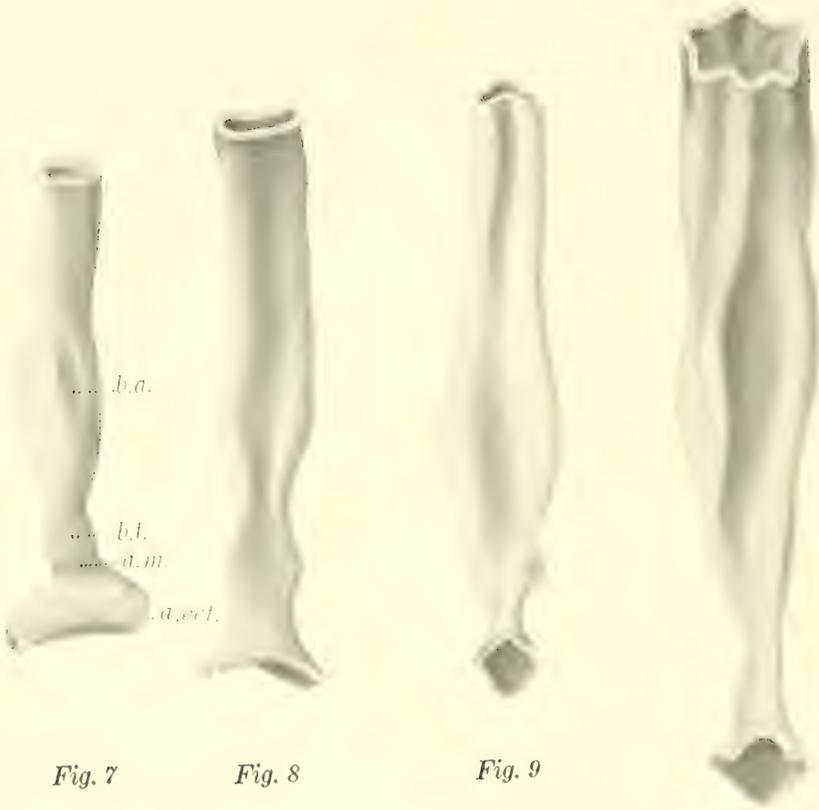


Fig. 7

Fig. 8

Fig. 9

Fig. 10

EXPLANATION OF FIGURES

- 7 to 10 Wax reconstructions of the epithelium of the pars analis recti.
 7 Human embryo of 22.8 mm. H.E.C., 871. × 36. *b.a.*, bulbus analis; *a.ct.*, anal ectoderm; *a.m.*, position of anal membrane; *b.t.*, bulbus terminalis.
 8 Human embryo of 30 mm. H.E.C., 913. × 36.
 9 Human embryo of 29 mm. H.E.C., 914. × 36.
 10 Human embryo of 37 mm. H.E.C., 820. × 36.

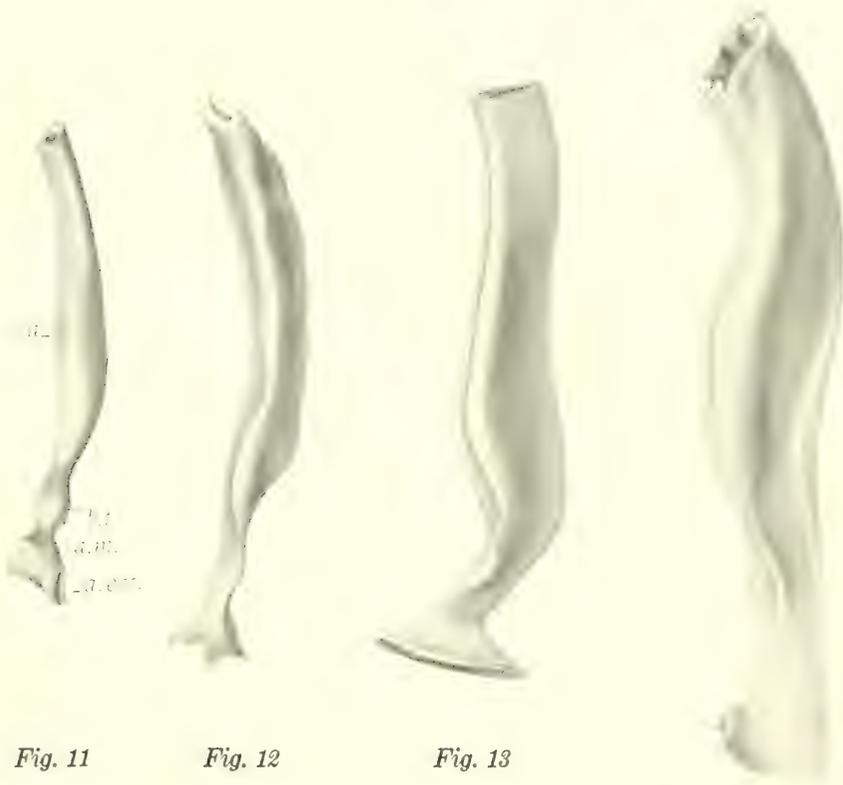


Fig. 11

Fig. 12

Fig. 13

Fig. 14

EXPLANATION OF FIGURES

11 to 14 Side views of the same. Abbreviations as in figure 7.



Fig. 15



Fig. 16

EXPLANATION OF FIGURES

- 15 and 16 Wax reconstructions of the epithelium of the pars analis recti.
15 Human embryo of 44.3 mm. H.E.C. 1611. $\times 36$.
16 Human embryo of 65 mm. H. 55. $\times 36$.



Fig. 17



Fig. 18

EXPLANATION OF FIGURES
17 and 18 Side views of the same.



Fig. 19

EXPLANATION OF FIGURES

19 Reconstruction of the epithelium of the pars analis recti of an embryo of 240 mm. \times 6.



Fig. 20

EXPLANATION OF FIGURES

20 Side view of the same.

PLATE 7

EXPLANATION OF FIGURES

21 Lower portion of the primary fold of a human embryo of 135 mm. $\times 72$. *gl.intrm.*, intramuscular gland; *sc.f.*, secondary fold; *tub.*, tubules; *x*, marks the point where the gland penetrates the internal sphincter muscle.

22 Intramuscular gland. Human embryo of 245 mm. $\times 72$. *a.*, acinous-like ending; *ep.w.*, epithelial wall.

23 Branched intramuscular gland. Human embryo of 245 mm. $\times 72$. *a.*, acinous-like termination; *amp.*, ampulla of gland; *br.*, branch; *ep.w.*, epithelial wall.

24 Sagittal section through the pelvis at birth. $\times 1$. *b.w.*, body wall; *bl.d.*, bladder; *C.I.*, 1st coccygeal vertebra; *ext.sph.*, external sphincter muscle; *l.a.*, levator ani muscle; *pl.tr.*, plica transversalis recti; *rec.a.*, rectal ampulla; *S.I.*, 1st sacral vertebra; *sym.p.*, symphysis pubis; *z.col.*, zona columnaris; *z.int.*, zona intermedia.

25 Sagittal section through the lower part of the rectum. $\times 6$. *a.v.*, anal valve; *r.c.*, rectal column; *rec.a.*, rectal ampulla; *sin.*, rectal sinus; *z.col.*, zona columnaris; *z.int.*, zona intermedia.

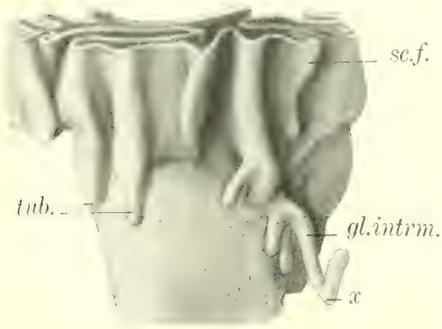


Fig. 21

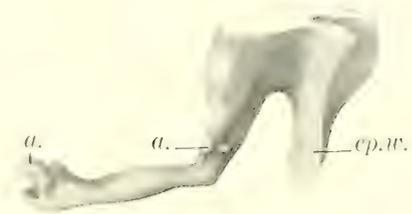


Fig. 22

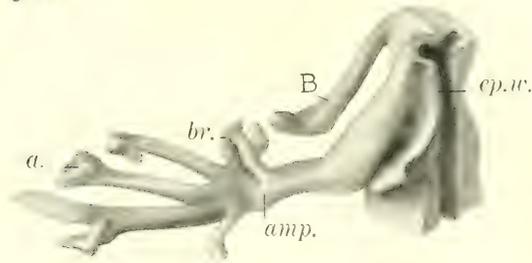


Fig. 23

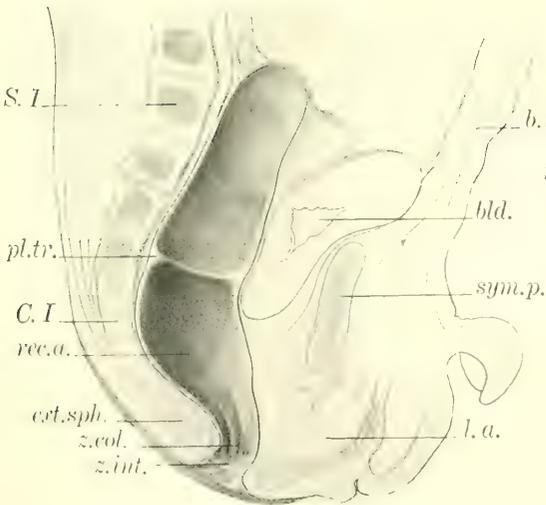


Fig. 24



Fig. 25

THE INTERSTITIAL CELLS OF THE MAMMALIAN OVARY: FELIS DOMESTICA

B. F. KINGSBURY

Department of Histology and Embryology, Cornell University

SIXTEEN FIGURES

It is a rather striking fact that while the occurrence of relatively large epitheloid cells, with clear vesicular nucleus, which are generally termed 'interstitial cells' has been known for a number of years to exist in the ovaries of the common mammals including man, they generally receive but scant mention in text-books of anatomy and histology. Herein accounts of the structure of the ovary usually contrast with those of the male reproductive organ, the testis, the interstitial cells of which are practically always fully described. On the other hand, contrasting with this conservative attitude we have the tendency on the part of many recent writers to see in these cells formers of an ovarian 'internal secretion,' even designating them as the 'internal gland' of the ovary. Yet, on the physiological as on the morphological side there is evident the same contrast between the definiteness of statement as to the interstitial cells of the testis and the vague and uncertain attitude as to the basis of the 'internal secretion' of the ovary.¹

¹ In recent works summing up results upon the "Organs of internal secretion," this contrast is revealed. Biedl ('10) concludes that the evidence strongly supports the secretory character of the interstitial cells of the testis, while stating that it is as yet inconclusive for the interstitial cells of the ovary. Swale Vincent ('12) quotes conclusions supporting the importance of the interstitial cells of the testis; he but incidentally mentions the interstitial cells of the ovary. Cushing ('12) says (p. 276): "In the testis there are two factors to be considered, the interstitial cells and the cells of Sertoli or spermatogenous epithelium. The former are undoubtedly related to the acquirement of secondary masculine characters of sex." Of the ovary he says (p. 279): ". . . . the interstitial cells of the gland which, in all probability, play a rôle similar to that of the cells of Leydig in the male. The existence of such cells in the ovary has been fully described by Limon."

A priori the recognition in the testis of specific cells as constituting morphologically a gland of internal secretion conveys the assumption of a morphologically comparable gland in the ovary. Indeed, the application to them of the term 'interstitial cells' carries with it the acceptance of their homodynamy with the interstitial cells of Leydig in the testis, which is provisionally adhered to in the present article.

The literature dealing with these cells of the mammalian ovary, specifically or incidentally, is extensive and is found in numerous papers on the structure and development of the ovary. From their mode of origin (discussed subsequently) more specific descriptions occur in connection with the theca folliculi, atresia folliculi, origin and nature of the corpus luteum, etc. Historical summaries have been well presented by a number of writers (Schulin '81; Harz '83; Limin '02; Schaeffer '11). A concise statement of the growth of knowledge of these cells will nevertheless be helpful at this point.

By Pflüger ('63) and by Schrön ('63) it would appear that these cells in the ovary were first described and figured, but His in 1865 was the first to describe them in detail (in the ovary of the cat) and discuss their significance. Their occurrence in the midst of the spindle-shaped stroma cells and an apparent close association with the blood-capillary network led him to regard them as developed out of the stroma cells (spindle cells) while at the same time he suggested a genetic relationship to the blood capillaries. Between these two mutually irreconcilable modes of origin of these cells he made no definite choice. He found them best developed in the 'membrana folliculi interna' (theca interna) which one is led to infer he believed developed out of them. He designated them as granule cells (Kornzellen) and recognized their lipid content, as did Pflüger. Waldeyer, in his classical paper on the ovary ('70) briefly mentions the 'Kornzellen' of His which he interprets as 'Wanderzellen.' Subsequently ('74) therefore in the paper in which he establishes his group of 'plasma cells,' although the interstitial cells of the testis were included in that group no mention is made of these ovarian cells. The next important paper dealing with these cells is

that of Tourneux ('79), earlier workers (Born '74; Slavjansky '79) seeing the cells but not identifying or discussing them. Tourneux compared them directly with the interstitial cells of the testis, applying to them—apparently for the first time—the same name. He compared them also with the cells of the suprarenal gland, coccygeal gland, carotid gland and the decidual cells of the uterus, adding them to the list of cells already grouped together by Mihalkovics ('73) and Waldeyer ('74). The term 'interstitial cells' (of the ovary) was immediately applied to them by MacLeod ('80) and has persisted up to the present. Some seven workers dealt with the ovarian interstitial cells between 1880 and 1898 when the papers of Kölliker, Clark and Rabl appeared and since then the interstitial cells of the ovary have received an increasing amount of attention. Four aspects of the historical growth of knowledge of these cells seem to the writer worthy of emphasis: (a) their relation to the tunica thecae interna and atresia folliculi; (b) their comparison with the cells of the corpus luteum (lutein cells); (c) their granular and lipid content; (d) their significance and their interpretation as constituting an 'interstitial gland.'

An association of the interstitial cells with the tunica interna of the follicular theca was recognized almost from the time of their first observation although the relation given was the reverse of that which subsequent observers determined to be the correct one. His ('65) made the tentative suggestion that his 'granule cells' give rise to this layer of the follicular wall. Harz ('83) inclined to the same view. Less emphatically or clearly stated, in the contributions of workers before 1898 (i.e., Paladino '88, Janosik '88, Schottländer '91, '93, Störkel, and others) the derivation of these cells from the internal theca of immature follicles undergoing regression, has been shown beyond all question for a large number of mammals of several orders—including man—through the investigations of Kölliker ('98), Rabl ('98), Clark ('98), Van der Stricht ('01, '12), Limon ('02), Allen ('04), Seitz ('06), Wallart ('07), Fellner ('09), Regaud and Dubreuil ('07) and others, some of these writers laying their emphasis on the atresia folliculi, others on the interstitial cells. It was to

be expected that in an organ as complicated as the ovary other modes of origin had been advanced. Hence we find the strands of interstitial cells interpreted as degenerating egg-tubes of Pflüger (Schulin '81) or derived from the Wolffian body (Balfour '78, Harz '84). From the medullary cords of the ovary in the fetal, new-born or young animal the strands of interstitial cells were not readily distinguished and the two structures were confused, notably by Balfour. Genetic relationship to the blood vessels was advanced by His and Mihalkovics. Tourneux and Janosik regarded them as connective tissue cells. Miss Lane-Claypon ('05) has more recently derived them from the germinal epithelium as cell cords with the potentiality of primordial ova. The appearance of the interstitial cells and their epitheloid arrangement led a number to assume an epithelial origin for them. Finally, their resemblance to the cells of the suprarenal organ (cortex) had been commented on as suggestive by Mihalkovics, Janosik, Limon, MacLeod, Van Beneden and Tourneux. It should be noted that while it is quite clear from the literature alone that the interstitial cells of the mature or maturing ovary come from the internal layer of the theca of degenerating follicles, those in the fetal or postpartum ovary can have no such origin. This will be discussed subsequently.

The relation of the problem of the interstitial cells to that of the corpus luteum is to be found in the view that has been more or less definitely expressed by many workers—that no fundamental difference exists between the regressive changes that occur in the ruptured and in the unruptured follicle when they undergo degeneration. First definitely expressed, as far as the writer knows by Schulin ('81) who believed he could find intermediate forms between the typical atresia and corpus luteum formation, the fundamental similarity of the two processes has been insisted on by Paladino ('88), Schottländer ('91), Hoelzl ('93), Kölliker ('98), Clark ('98), Bouin ('02), Allen ('04), Van der Stricht ('02), Limon ('02), Fellner ('09) and others. The atresia folliculi has been designated as a corpus luteum atreticum (Kölliker, Van der Stricht), false corpus luteum (Schottländer and others), while by Fellner the interstitial cells are called

'theca lutein cells'—a name carrying with it the interpretation of similarity but not genetic identity. Entirely comparable is Pinto's 'Paralutein cells.' On the other hand, the homodynamy or comparability of structures arising out of the ruptured and unruptured follicles is emphatically denied by many—notably of course, Sabotta—and the problem on this side merges with that of the origin and significance of the corpus luteum. The comparison made by Bouin and Ancel is considered subsequently.

One of the most striking characteristics of these cells is their granular content of a lipoid nature. Recognized by Pflüger and His, this has been commented on by most later writers. Degeneration products derived from the follicular epithelium or from the theca; unutilized nutritive material; nutritive material for the developing ova; secretions, etc.; these are among the interpretations made of their presence, but no decisive evidence has been brought forward in favor of any one of these views. A yellow or orange pigment (a lipochrome) may or may not be present, with or without the lipoid granules, seemingly somewhat characteristic of some forms and of certain periods of life in other forms. A rather close association of pigment and lipoid appears to exist, the condition in the interstitial cells clearly paralleling closely that in the corpus luteum.

A number of recent writers have united the interstitial cells of the ovary under the collective name of 'interstitial gland' (Bouin '02, Limon '02, Regaud and Dubreuil '07, Aimé '07, Wallart, '07, Bianchi '07, Mulon '10, Van der Stricht '12). The term has been objected to by some (e.g., Fränkel '05, Bühler '06) as has the assumption of the secretory character of these cells. Fellner accepts the secretory interpretation without making use of the term 'interstitial gland.' It will be necessary to discuss this interpretation subsequently.

The different mammals in which ovarian interstitial cells have been described and their origin and significance considered comprise a relatively large number of species, almost exclusively from the orders of Chiroptera, Insectivora, Rodentia, Carnivora, Ungulata and primates—particularly man. To the ovary of the rabbit, guinea-pig, bat, cat and man most attention has

been devoted. The conclusions in favor of the 'interstitial gland' interpretation have perhaps been largely based on the conditions in the rabbit and bat. Only a few observations have, therefore, been made as far as known to me on the lower orders of mammals (marsupials and edentates) by Benthin ('11) and Schaeffer ('11). Within the range of animal forms examined, interstitial cells are present in very varying numbers. Thickly massed in the rabbit, they are present in large amount in the cat's ovary, scantily developed in the human ovary, they appear to be practically absent in certain other forms, such as the pig, sheep, cow. In general, it would seem that they are abundantly present in the small mammals examined and few or absent in the larger mammals in the adult.

The great variability in their development certainly calls for careful consideration in drawing any general conclusions as to their significance, as has been insisted upon by Fränkel ('05). Furthermore, it would appear that the same variability is found in the period of life at which the interstitial cells are present. Upon this basis Aimé divided the mammals into four groups: (a) those in which the 'interstitial gland' occurs in fetal life only (e.g., the horse); (b) those in which the 'interstitial gland' is present only in postpartum (essentially, adult) life (Chiroptera, Insectivora, rodents); (c) forms possessing *no* 'interstitial gland,' wherein he included man, pig, sheep, goat, boar, dog; (d) mammals in which an 'interstitial gland' occurs both antepartum and postpartum, as an example of which group he gives the cat. It might be added therefore that Aimé accordingly distinguished two groups of interstitial cells or two forms of 'gland:' (a) the fetal, derived from the 'connective tissue;' (b) the adult, derived from the theca interna. This grouping has been essentially followed recently by Popoff ('11). Any discussion of the strict applicability or value of these distinctions will not be entered into now. It may be pointed out however that a full study of the developmental history of the ovary and of the interstitial cells in particular, has been made in no form save the cat, by Saimont ('05) and v. Winiwarter and Saimont ('08).

The ovary of the cat is well suited for a study of the genesis and significance of these cells by reason of their abundant presence and discriminate arrangement, as well as because of the availability and convenient size of the material. It is therefore rather interesting that it was apparently in this form that the interstitial cells were first seen and described by Pflüger in 1863, figured by Schrön in the same year, and discussed by His in 1865 (his Kornzellen). The interstitial cells of the cat's ovary have since received attention from Creighton ('78), Harz ('83), Janosik ('85), Plato ('97), H. Rabl ('98), Coert ('90), Ganfini ('07), and Saimont and v. Winiwarter and Saimont as above mentioned.

My interest in these cells dated from 1908 when I began an investigation to satisfy my curiosity as to their structural characteristics and mode of origin in the cat's ovary. The material collected and made use of in this study comprises 63 complete or partial sets of serial sections through the ovary, from the fetus (9 series), after birth (25), before sexual maturity (15), before, during and after pregnancy (i.e., in lactation) (14). Most of these series illustrating well the general morphogenesis of the cat's ovary a special study of this aspect of the development of the cat's ovary was made and has been separately published.² Indeed, it soon became apparent that any explanation of the interstitial cells was intimately linked with that of the developmental processes taking place in the ovary as a whole, and that they could not be separately interpreted.

A brief statement of the general features of the morphogenesis will facilitate the discussion of the interstitial cells subsequently. The ovary in the cat increases mainly by peripheral growth, the ultimate source of the germ cells and indifferent or follicle cells being the mesothelial covering.³ These and the stroma ovarii

² B. F. Kingsbury: The morphogenesis of the mammalian ovary (*Felis domestica*). *Amer. Jour. Anat.*, vol. 15, no. 3, pp. 345-379. November, 1913.

³ This is stated with the reservation that the ultimate source of the germ cells may be from the entoderm, in accordance with the evidence therefor adduced by several recent writers. The stromal cells appear to be derived from the mesothelial covering (cf. Allen '04; Whitehead '04; Rubaschkin '12). This origin, I believe, would not affect their essential connective tissue nature.

constitute the characteristic tissues of the ovary. The earliest parenchymal growth—as usually termed, the medullary cords, constituting the first proliferation—occupy therefore the center and mediastinal portion of the ovary. The medullary cords at first are free from distinguishable germ cells, although later definite ova seem to appear in their midst. Later proliferations from the surface epithelium produce cell cords in which the reverse of the conditions characteristic of the medullary cords holds—the germ cells become the dominant cells and the indifferent cells are distinguishable with difficulty. The differential growth of the ovary is centrifugal, and hence older stages of the oogenesis are more centrally located. The zone of oogonial multiplication is peripheral, the proliferation of new oocytes ceasing soon after birth. Connection of the egg cords with the surface epithelium becomes lost two to three weeks after birth, and by separation of the oocytes composing the outer portions of the egg cords, resting or primary follicles are formed, marking out a definite cortex. While the peripheral growth changes are leading to the establishment of the definite cortex, within the medulla the differentiation takes the form of follicle-formation. The first follicles are apparently formed within the medullary cords themselves, later ones include ova derived from the inner portions of the egg cords. The early or medullary follicles are very irregular and largely pluri-ovular. The follicle formation follows the general plan of centrifugal differential growth, so that, in general, less advanced follicles are more peripherally located. Many of the large, irregular pluri-ovular follicles attain a marked development so that in old kittens with the greatest development of these follicles the ovary reaches a size greater than at a subsequent period, since these, as well as all the early formed medullary follicles degenerate. Such degeneration is particularly profound as sexual maturity is approached, although degenerations are constantly present in the ante- and postpartum periods, in less mature ovaries, and subsequently throughout the period of sexual maturity. The degenerations of the pre-sexual period are of a somewhat different type. The early medullary follicles degenerate; later ones that attain a large size as

Graafian follicles are exceedingly irregular, atypical, and ultimately undergo atresia. The follicular growth processes and conditions are different in the immature ovary, itself undergoing rapid growth. In the growth of the ovary the stroma obviously plays an important part, and in no wise is to be thought of as playing a passive, purely supportive rôle.

In the above exceedingly brief outline of the morphogenesis of the ovary I have presented the conclusions reached in a study of the later development of the ovary. In general there has been full confirmation of the monographic investigation by v. Winiwarter and Saimont. In certain respects, however, the emphasis, point of view, or interpretation has been different. These matters of divergent interpretation have been discussed in the previously published article on the morphogenesis.

The growth of the ovary is a continuous process, and the recognition of periods is rather artificial; however, it is convenient to distinguish (a) period of early (1st) proliferation; (b) the period of later (2d) proliferation; (c) postpartum period—up to the establishment of definite cortex of resting follicles; characterized by development of medullary follicles; (d) the presexual period; growth of the pluri-ovular large medullary follicles; (e) the period of profound degeneration; (f) sexual maturity; (g) senescence.

The interstitial cells first make their appearance during the second period, being largely associated with the medullary cords, and hence central. Small groups of them, however, occur constantly in the stromal septa between the egg cords in the primitive cortex (figs. 1 and 2). From this period on, the interstitial cells are present in the ovary of the cat into extreme old age. How early they are present in the fetal ovary I cannot say. The youngest ovary in which they were identified was from a 95-mm. fetus. Inasmuch as the series of younger ovaries were not prepared by a method¹ that rendered their identification

¹ Two methods were used with advantage: (a) Flemming's fluid (Hermann's or Benda's) fixation, the chloroform paraffin imbedding method, the sections being mounted unstained and without coverglass under a thin film of chloroform balsam. In toto staining with carmine might, I believe, have been advantageously em-

easy (Flemming's fluid fixation, the sections bleached and stained with iron hematoxylin) some few interstitial cells may have been present and not detected. This is easily possible from the mode of their formation. Saimont ('05) has described and figured them as occurring in the basal connective tissue nucleus and in the stromal lamellae between the medullary cords in fetuses of 29 to 52 days age (ca. 25 to 91 mm. length). He has recognized in the cat's ovary three periods of development of interstitial cells, (a) the period just referred to; (b) a second period, from about 58 days postcoitum (ca. 120 mm. length) to sixty or sixty-seven days postpartum, in which they occur in the neighborhood of the *rete* and in the zone of the medullary cords; and (c) a third period, extending from about 50 days postpartum into adult life in which they occur as the large thecal cells in follicles in process of development (*en voie de développement*). The interstitial cells of the first period seem to completely disappear before the second period sets in. These cells I have not studied, and hence cannot discuss their mode of origin. The distinction of the second and third periods is one of convenience only. As will appear subsequently my conclusions as to the origin of cells of the 'third period' and the interpretation for all periods are quite the reverse of Saimont's.

As to the origin of the interstitial cells, the statement of my conclusions is brief. They arise as a modification of stroma cells, and the stroma is therefore the parent tissue for this type of cell. This conclusion is but a confirmation of the results of nearly all later workers upon the subject (Tourneux, Allen, Janosik, and others).

By increase in the amount of cytoplasm and the appearance of free lipid therein, the typically spindle shaped stromal cell increases largely in size, and is altered in shape, becoming thus recognizable as an interstitial cell. The correspondingly elongated and densely staining nucleus enlarges to a spherical form,

ployed in this method. The lipid granules are completely preserved by this technique; (b) fixation in modified Zenker's fluid (1 per cent of acetic, or less), followed by a dichromate mordantage, paraffin imbedding, and a Weigert hematoxylin stain, as described in the *Anatomical Record*, vol. 5, p. 313.

clearer, with a well defined 'nucleolus.' No special cytological study of nucleus or cytoplasm was made, hence the nature of this nucleolus was not ascertained, whether true or false.

The problem of the interstitial cell, on this side, therefore, resolves itself into an examination of the conditions under which this transformation occurs, and the part that the stroma plays in the ever-continuous processes of growth within the ovary.

That the stroma plays an important part in the development of the ovary as a whole, in the transformations that take place in the medulla of the developing ovary, and in the phenomena of follicle development in the adult ovary, becomes quite evident from the examination of such a series of ovaries as has been at my disposal. The histological growth changes are so complex however that their analysis and broad comprehension are exceedingly difficult. Stromal strands and lamellae penetrate the primitive cortex, but any particular correlation with the egg cords is not apparent. Oocytes that have either earlier or later become separated by the breaking up of the egg cords, are surrounded by a follicular epithelium, and there is usually recognizable a concentric stromal investment. In the primary follicles of the cortex in the adolescent and adult periods, the follicular epithelial cells are so thin that careful examination is necessary for their recognition. Such follicles possess no evident theca. As soon, however, as the growth of the follicle begins, and the follicular epithelium becomes cuboidal, columnar, and then stratified in the well known method of growth of a Graafian follicle, a concentric arrangement of the stroma is evident, becoming as growth proceeds, the theca of the Graafian follicle. The so-called medullary cord of the fetal and post partum ovary possesses a stromal sheath as a form of theca (figs. 11 and 12), which becomes very evident in the larger masses. The concentric arrangement of the stroma is found whether an ovum is enclosed or not. Naked ova, lying free in the stroma are not found save occasionally. Such ova are apparently always degenerating or degenerated. The growth correlation which leads to the formation of a definitive theca in the development of a Graafian follicle, appears to be more directly between stroma and

the indifferent or follicle cells rather than directly between stroma and developing ovum.

During the first three months after birth great irregularity characterizes the growth changes of the parenchyma within the interior of the cat's ovary, causing morphological relations of considerable complexity and irregularity, which are differently interpreted by myself (in the previous paper) and by v. Winiwarter and Saimont ('08). The series during this period indicate that it is one of marked growth of the stroma as well as of the parenchyma so that it seemed to me the irregular and atypical structures expressed, morphologically an attempt at follicle formation during rapid growth of the ovary as a whole. While the stroma contributes to the general complexity, a relation to the masses of indifferent cells and irregular follicles, is nevertheless evident, as appears in some of the photographs illustrating the previous paper.

Many of these follicles develop into the large, irregular pluriovarular follicles previously mentioned. These possess very definite thick stromal thecas, including, in fact, most of the stroma in this zone of the ovary.

The period of developing medullary follicles is likewise the most complex in the distribution of the interstitial cells, which occur in smaller or larger masses in the midst of the stroma strands, which show in general the same relation to the follicular masses as does the stroma out of which they are developed.

The zones occupied by the bulk of the interstitial cell groups in four typical stages—6 days, ca. 5 weeks, ca. 3 months, and in the adult ovary—may be illustrated by figures 1 to 4, in which the dense black of the osmic acid stained lipid locates the interstitial cells in which it is contained.

It may be noted that the interstitial cells occur in the zone where growth (of the indifferent cells) is apparently most active—about the medullary cords, before and after birth; in the zone of the medullary follicles, during their stages of growth, etc.

Hence it might appear that they had to do particularly with those growth changes and possessed a trophic 'function.' This was the interpretation of Saimont ('05) and of v. Winiwarter

and Saimont ('08); but I believe when closely examined, the evidence for this interpretation is not only inconclusive, but on the contrary points in quite the reverse direction. This will be discussed presently.

Before taking up the question of the interpretation of these cells, I wish to confirm the conclusion of Saimont and others that these cells are transeient structures in the ovary: that they, as interstitial cells, possess no permanency; that they come and go. This conclusion would be a priori self evident from their mode of origin in the adult ovary. I further agree with Saimont that their fate is not one of degeneration, but that they revert to the cell type out of which they were developed, that is, the stroma cells. Saimont accordingly recognizes (a) young interstitial cells, (b) transitional forms, (c) adult interstitial cells, (d) degenerated interstitial cells; i.e., those reassuming their character as stroma cells, and (e) hypertrophied cells. These stages, he finds to be somewhat characteristic of the three periods that he recognizes. Thus the stage (a) he thought limited to the first period; the stage (b) occurs only after birth, etc. It might be pointed out, however, that, obviously, from their very evident continous formation, when once fully established, essentially all stages must exist in the ovary at the same time—as in the adult. The distinction of stages is but a matter of convenience in description, and the terms are likewise of but metaphoric value.

The interstitial cells thus possess no morphological individuality and hence do not, strictly speaking, deserve recognition as a distinct kind of cell. They are stroma cells, which under certain conditions, undergo changes, the most striking and characteristic histological alteration being the accumulation of lipoid granules within their cytoplasm. Apparently with the passing of these conditions they again become stroma cells.

In considering the question of the interpretation of these cells, therefore, the above definition must be kept in mind, since what may be true of a stroma cell need not necessarily be also true of an 'interstitial cell.' This is particularly true of the 'trophic function' interpretation as advanced by Plato and accepted by

Saimont ('05). As has been indicated the conditions within the ovary during growth might well be considered to support this interpretation. The morphological relations during growth are of considerable complexity whereas during the adolescent and adult period (Saimont's 3rd period), the genetic relations of these cells are of the clearest, and show conclusively that these cells arise from the theca interna during the degeneration of Graafian follicles as atresia folliculi. During the growth of a follicle from the primary resting follicle to the appearance of the antrum, the cells of the theca, which becomes progressively more distinct, retain their spindle shape and the general histological appearance of stroma cells. They are almost or quite free from demonstrable lipid. Next the follicular epithelium the cells are more densely arranged and larger (theca interna). In Graafian follicles of different sizes and degrees of development, the theca interna cells are largely free from demonstrable lipid. Frequently, lipid granules occur and in some cases, particularly in the larger follicles that are evidently nearing maturity, the amount of lipid granules within the cells of the internal theca is quite marked. The cells, while somewhat larger, still retain their spindle shape which is doubtless largely an expression of the mechanical conditions under which they exist. Such follicles showing lipid content in the theca cells are apparently normal. It is this, I believe, that led Saimont to ascribe to the interstitial cells a 'trophic function.' The amount of free lipid in such cells is small and they do not correspond to his figures of 'adult' cells.

With the onset of atresia, a succession of changes occurs that markedly increases the fat content in the theca cells and determines their transformation to the so-called interstitial cells. From a series of nine photographs showing successive steps I select four stages to illustrate atresia folliculi (figs. 5-8). So well have the salient histological features of atresia folliculi been described, for the cat particularly by H. Rabl, that any extensive description is unnecessary. One of the earliest indications of the onset of degeneration is an alteration in the liquor folliculi. Instead of coagulating on fixation as a loose flocculant mass, it

is more granular, seemingly denser and becoming more dense and homogeneous as the atresia progresses. The ovum early shows an alteration, loses its spherical shape, becoming ellipsoidal, as though affected by an increased intra-follicular pressure. The follicular epithelium and theca, in early atresia show in the cat no marked alteration. In later stages the follicular cells become markedly spindle-shaped or stellate. Lipoid granules which (in the cat) are only scatteringly demonstrable in the follicular epithelium become more numerous, typical karyolysis sets in, of the type early described by Flemming in this kind of cell as chromatolysis, and the cells subsequently completely disappear. There is an undoubted 'invasion' of stromal connective tissue and possibly of leucocytes as well. The liquor becomes completely absorbed, and the antrum thus obliterated. The egg cell disappears, the egg membrane (zona pellucida) persisting for a long time (fig. 8). The last recognizable trace of the follicle is the irregular ring of interstitial cells derived from the theca interna. Ultimately this arrangement is broken up under the stress of the growth changes within the ovary, and there are but isolated groups of interstitial cells dispersed apparently at random in the stroma. These finally, according to the observations of Saimont and myself, revert to their original stromal cell form.

The relation of the interstitial cells to the atretic follicles is strikingly shown in preparations in which osmic acid fixers, such as Flemming's fluid, are used. The reproduction of typical sections is given in figures 4, 9 and 10, from which, as in figures 5 to 8, of which they give the complementary 'lipoid picture,' the relation of the interstitial cells to atresia is illustrated. Several degrees of atresia are there shown.

So consistently has this mode of thecal origin of the interstitial cells of the adult ovary been supported by the observations of nearly all who have devoted careful study to this phase of the subject, that it may be accepted as clearly proven.

I find therefore no support for the interpretation of Saimont that (in his 3rd period) the interstitial cells are found as "large thecal cells in follicles that are undergoing development," but

that they are the thecal stroma cells of Graafian follicles that have undergone or are undergoing degeneration. Saimont seems to have based his conclusions upon the conditions in the theca of the larger Graafian follicles. In atresia there are formed as interpreted by him, hypertrophied interstitial cells. Reproduction of such cells shown in figures 13 and 14, however agree rather with his figures of 'adult' cells. He published no figures illustrating specifically this period, nor have v. Winiwarter and Saimont as yet published descriptions or figures of the follicle formation in the adult cat's ovary. If it were stated that the thecal cells were intimately associated with the growth changes of the maturing follicle and it were considered advisable to so express this by ascribing to them a "nutritive or trophic function" there would, it seems to me, be little basis for a disagreement, since there is much that indicates the importance of the stroma in the growth processes within the ovary. The interstitial cells might thus prove to be an expression of the arrest or abeyance of a trophic function.

Whatever may perhaps be ultimately shown to be the exact nature of the correlation, the phenomena of atresia show that the interstitial cells stand as an expression of an altered metabolism associated with the (degenerative) processes that occur within the atretic follicle, although the occurrence of free lipid in thecal cells of apparently normal follicles indicates both the delicacy of the metabolic balance between theca and follicular epithelium and that the appearance of free lipid in the thecal cells is not necessarily an indication of irreversible degeneration.

The ovarian parenchyma (egg cells and indifferent or follicle cells) is characterized by the high evident lipid content of the cells. Oocytes of the primary resting follicles show little or no free lipid with osmic acid as an indicator. The application of a mitochondrial technique, however, reveals the numerous mitochondrial granules which undoubtedly represent lipid in masked or combined form. With the growth of the ovum numerous globules of free lipid make their appearance in the cytoplasm. The follicle cells of normal follicles show little or no free lipid. Mitochondria are however present in abundance, particularly

in the cytoplasm toward the ovum in the cumulus, or toward the antrum in other portions. With the degeneration of the ovum in atresia, numerous lipid granules appear in the follicle cells.

In the fetal and postpartum ovary lipid granules are abundantly present in the medullary cords. They were recognized first in my series in the 95-mm. fetus and from that time on appear to be constantly present in the indifferent cell masses which do not immediately surround an ovum until such disappear from the ovary before sexual maturity. In the period of development of the peculiar medullary follicles (ca. 5 weeks), it is interesting to observe that in the irregular follicle, such as shown in figures 18, 24 and 25 in my previous paper, the epithelium immediately surrounding the ovum is particularly clear from such free lipid, whereas the follicular epithelium of the remainder contains it in abundance. This is easily demonstrable by means of osmic acid fluids, and in preparations fixed and stained by the Weigert hematoxylin method, the portion containing the greater amount of lipid stains much more intensely than the sheathing follicular cells. In the 3 months ovary (fig. 3), containing the large irregular pluriovular follicles, the follicular epithelium bordering the antrum and ova mainly lacks the free lipid content. Many of these Graafian follicles however possess irregular tubular extensions of the follicular epithelium, and the follicular cells composing these contain free lipid droplets.

It is in the medulla in particular association with the free lipid containing medullary cords and irregularly developing medullary follicles, that the groups of interstitial cells are found. Figures 11 and 12 show medullary cords of a 3 to 4 day kitten, illustrating the stromal investment, the 'loose' character of the cells and the free lipid content, some of which had doubtless been dissolved out before it was drawn, since it was from a stained and covered series (No. 11). They likewise show the modified, free lipid-containing stroma cells, or in other words, interstitial cells, and their close relation to the cells of the medullary cord.

During the period of growth of the medullary follicles, to the interpretation of which considerable discussion was devoted in

my previous paper, the relation of the interstitial cells to the follicular masses is likewise evident, although much more intricate and less exact, due in part, I believe, to the shiftings during a period of marked growth of the ovary as a whole. In illustration of the relations at this period two line drawings (figs. 15 and 16) are introduced from which the location of interstitial cell groups may be seen.

Finally, from figure 3, the location of the interstitial cells in the period of large irregular pluriovular follicles may be seen. It will be noted that the theca of these follicles contains abundant lipoid containing cells. These follicles are quite atypical, aside from their irregularity and pluriovular character, in the thinness of their follicular epithelium, and as v. Winiwarter and Saimont pointed out, in the 'abnormal' appearance of contained ova, one element of which, the scanty amount of lipoid contained, interests us here.

From the above it may be seen that the follicular growth processes within the medulla of the immature ovary depart widely from those of the adult period, and it is undoubtedly in correlation with the irregular and essentially atypical ('abnormal') character of the growth processes rather than with growth itself that the interstitial cells make their appearance. In my previous paper, I have interpreted the growth relations encountered as essentially successive 'attempts' at normal follicle formation, becoming more typical as the presexual development of the ovary advances. All these early formed follicles, as v. Winiwarter and Saimont first recognized, are doomed to degenerate. There succeeds the period of large irregular pluriovular Graafian follicles a period of profound degeneration, and it is at this presexual period that the cat's ovary contains the greatest relative number of interstitial cells. From an ovary of this period figure 9 is taken, and for it might have been substituted reproductions of sections from several ovaries in which the mass of interstitial cells was much greater.

No fundamental difference is thus felt to exist between the conditions of interstitial cell formation in the adult and in the growing ovary.

In the fetal (112 mm.) and newborn kitten small groups of interstitial cells were mentioned earlier in this paper as occurring in the stromal lamellae between the egg cords of the primitive cortex. Similar small groups occur in the primitive cortex of older kittens. In the adult in the definitive cortex (zone of resting primary follicles) I did not find undoubted instances of their presence. I have been unable to find any clear correlation of these with growth or degenerative processes. Correlations doubtless exist which continued study of the changes in the primitive cortex may reveal.

My observations do not support the conclusions of Aimé and of Popoff, that there are two morphologically distinct 'interstitial glands,' the one fetal and medullary in position, the second adult and 'cortical,' that is, formed in the atresia folliculi; the first possibly, as Popoff believes it to be, homodynamous with the testicular interstitial apparatus, corresponding to a medullary cord—seminal tubule homodynamy. I cannot ascribe to the interstitial cell groups any morphological value, as is evident from the consideration of them given above.

I desire next to consider briefly the appropriateness of the term so generally used by a number of recent writers—that of 'interstitial gland.' The term 'gland' is avowedly employed somewhat indiscriminately and subject to abuse, particularly as applied in the group of 'internal secretory glands,' or 'endocrine glands.' The term may be used in a morphological or in a purely physiological sense. In the morphological sense, namely, as a differentiation of definite cells in development for the elaboration of specific chemical substances, and possessing accordingly corresponding characteristic structure, morphological arrangement and relations—the interstitial cells of the ovary certainly do not deserve the designation of gland. They are clearly more or less transient transformations of stroma cells or theca stroma cells under altered conditions of metabolic correlation.

There are involved here somewhat the same considerations that led Kohn ('00) to reject the name as applied to the various groupings of phaeochrome cells.

There is no constant or characteristic arrangement of the interstitial cells, nor does there appear to be any marked or peculiar relation to the blood or lymph vascular system. This is however a distinctly disputed point. Since the time of the paper of His, a peculiar relation of the interstitial cells to the blood vessels has been described by Limon, Aimé, Athias and Saimont. This, on the other hand, is denied by Cohn.

Perhaps the most suggestive evidence of a glandular arrangement of the interstitial cells and a definite relation to the vascular system is that presented recently by O. Van der Stricht ('12) in his studies of the ovary of the bat, *Vespertilio*. He adduces arguments and evidence to show that the 'secretion' finds its way into the lymphatic channels and therein is transported from the ovary. I fail to find in the cat any unusual or peculiar relation of the interstitial cells to either the blood vessels or lymph vessels.

In fact, the tunica thecae interna seems to become markedly less vascular in atresia folliculi. Two conditions should I think be borne in mind in considering this phase of the subject: first, that the theca interna of the maturing Graafian follicle is markedly vascular—in very obvious correlation with the growth of the follicle, so that the thecal cells naturally possess a quite vascular environment; second, that in atresia folliculi a relatively large amount of fluid and products of histolysis must leave the ovary by some channel, and in some forms and at growth periods where the degeneration was extensive, a great secretory activity on the part of the interstitial cells might be simulated. In the reversion of the interstitial cells to the stromal cell type also a relatively large amount of substance must pass from them without necessarily possessing any ulterior significance as a secretory phenomenon.

The great variability in the presence and development of the interstitial cells in different forms is a third count against their possessing any morphological value as a gland. This evidence appears clearly from the studies of Aimé, Fränkel, Schaeffer and others. I cannot see any escape from the force of Fränkel's argument. A gland existing for the specific 'purpose' of forming

substances of very distinct value to the organism as a whole, would be as constant in its presence and development.

Most biological problems, particularly those attempting to explain the genetic significance of a structure or organ, possess two quite distinct aspects: (a) the determination of the processes that underlie the structural appearance, their correlations and explanation in terms of cause and effect, (b) the part they play in the bodily economy—for the individual or the race; as commonly expressed, the 'purpose' for which they exist, their 'function' in the organism; as the writer prefers to put it, their contribution to the complex pattern of the bodily activities and whose dominant component is adaptation. The existence of the double character of developmental problems and the significance of this, is not, I believe, sufficiently appreciated at the present day. If it is possible to ascribe a 'function' to an organ, the end and aim of its investigation is frequently believed to be attained, without full comprehension of what has been gained in the determination of 'function.' It is largely due to the dominance of the conception of the animal organism as a colony of organs each with its specific function or functions, that the interstitial cells are seized upon as an internal secretory gland, because there are no other cells in the ovary which meet the requirement of 'gland cells.' It is I believe the analogy of a superficial resemblance that here, as in other instances, has led to a false morphological grouping.

Apart from the question of the recognition of the interstitial cells as a gland in the morphological sense, is the quite distinct question as to whether they constitute a gland in the physiological sense; that is, whether from them, as interstitial cells, come substances formed as a result of their interstitial cell metabolism, which reaching the circulation, produce characteristic effects in other parts of the body. Any adequate consideration of this question lies beyond the scope of this paper and outside any morphogenetic study of the ovary. I desire therefore simply to offer a few comments upon certain aspects only. The striking characteristic of the interstitial cell is the large lipoid content. This apparently consists of phosphatids, fats and cholesterol,

together with a lipochrome (lutein).⁵ The suggestion is close therefore that the 'lipoids' are either closely concerned with the ovarian effect upon other parts of the body, or otherwise important. We have thus the interpretation of Regaud and Dubreuil that the secretion is lipid in nature; and that of Loisel that the lipid is associated with an activity in the neutralization of poisons formed in the bodily metabolism. He therefore looks with favor on the comparison of the interstitial cells and the cells of the suprarenal cortex made by Mihalkovics and others. As a source of 'internal secretion' he considers the degeneration of egg and follicle cells as well as the interstitial cells, and if I interpret him rightly, the undegenerating follicle and egg cells as well. Mulon ('10) holds similar views of the anti-toxic action and of the comparison with the suprarenal cortex.

However attractive the view that the interstitial cells are the formers of specific substances that are correlated with the appearance of the secondary sexual characters, it should be borne in mind that there is no direct evidence in favor of the view, nor does it seem possible from the character of their morphological position and relations that such evidence could be obtained. The circumstantial or indirect evidence seems to the writer, far from conclusive on this point. The following comments are offered from a purely morphological point of view. First, the same argument holds against the acceptance of these cells as constituting an internal secretory gland in the physiological sense as against their recognition as a morphological gland, namely, the variability in the amount and in the time of appearance, mentioned and briefly discussed above and in the introductory paragraphs of the paper. In many forms, after birth at least, the interstitial cells appear to be lacking; in others lacking before a certain age (e.g., rabbit). It should of course be borne in mind that a full history of the development of the ovary in most of these forms has not been worked out. In the cat, from my series, interstitial cells appear to be continuously present

⁵ Cf. Regaud et Dubreuil; Pargon, Dumitresca and Nissipesco, Wallart; Hohn and Staedeler; Mulon.

in the ovary from a 95-mm. fetus to extreme old age, and Saimont has described them in younger fetuses, stages that I lacked. They are markedly abundant before the time of sexual maturity, and are likewise abundantly present during adult life. The number of series is too limited to determine whether they are more abundant during pregnancy, but have given no indication that such is the case. The large numbers of interstitial cells in the ovary of the maturing kitten might be suggestive, were it not for their presence in both ovaries of a 17-year-old cat. One ovary was atrophic, oocytes had completely disappeared, but interstitial cells were relatively abundant as were cords and groups of the indifferent or follicle cells. The other ovary was hypertrophied, due to the large increase in the indifferent cells giving a superficial resemblance to a cryptorchid testis. Interstitial cells were present in relative abundance. Hence the distribution throughout the different periods of the life cycle gives no support to their secretory nature.

The stromal cell might with more likelihood be seized upon as the former of ovarian 'hormones' than the interstitial cell which is but a transitory development out of it of variable occurrence. Still more likely, from the writer's point of view, if he may be pardoned for intruding it, is the suggestion that end-products of the metabolism of the parenchyma itself may be a source of 'hormones' upon which depends the effect of the ovary upon the bodily metabolism. These possibly are set free in the degeneration of ovum and follicle.⁶ Certain it is that one of the most striking features of ovarian morphogenesis is the constant degeneration of egg cells and follicle cells throughout fetal and postnatal development until the onset of sexual senility. Before the onset of sexual maturity the degeneration is most pronounced (cat, man). The assumption that the end-products of metabolism may stimulate growth, is not I believe opposed to the facts of general physiology but rather the reverse. Whatever may be the outcome of future investigations as to the source and nature of the substances through which the ovary affects

⁶ Compare Loisel, 1905, p. 89.

the organism, the study of the origin of the interstitial cells indicates clearly that considerable hesitancy should be observed in declaring them specific glandular elements.

The corpus luteum and the corpus atreticum have been repeatedly compared, and despite marked differences there appears to be much fundamental resemblance. Both express essentially reactions of degeneration. In both relatively large amounts of lipoid appear in their cells attended by the presence of lipochrome. In the one it is the follicle cells that apparently undergo hypertrophy—although this is still a disputed point, and it is easily conceivable that the process may differ in different forms. In the other the cells of the theca alone hypertrophy and become charged with lipoid. The older describers of the corpus atreticum compared it with the corpus luteum—sometimes perhaps confused the two—or described intermediate forms (Schulin '81). Bouin and Ancel have more recently revived the comparison, placing it on the modern 'functional' basis of the internal secretions. The mammals are divided by them into two groups: those in which ovulation is spontaneous, at definite periods, and those in which it occurs at coitus. Those of the first group possess, accordingly, two varieties of corpus luteum, a corpus luteum periodicum (menstruationis), and a corpus luteum gestativum (graviditatis), while the forms of the second division have but the last kind of corpus luteum. These forms possess, however, an 'interstitial gland' which takes the place of the corpus luteum periodicum and is not present in those mammals which possess the two varieties of corpus luteum, among which Bouin and Ancel include man, dog, cow, mare, sow. Both classes have thus two kinds of internal secretory gland; the first associated with the uterine changes at pregnancy, the second subserves other forms of ovarian influence.

While there may be an ultimate significance in the comparison of corpus luteum and corpus atreticum, on the morphological side this particular comparison fails, if for no other reason, because in one member of the forms of the first group (man), interstitial cells are present, and in the other forms it is quite clear that corpora atretica occur and differ in no fundamental

characteristics in their formation Benthin ('11); Schaeffer ('11). There have been a number of investigators whose work has shown clearly that in man atresia folliculi, and interstitial cell formation follows the same plan as in the cat, for example. Grohe ('63) showed that before puberty (Graafian follicles were continuously formed. Slavjansky ('74) described atresia in ovaries from childhood, the sexually mature and during pregnancy. Schottländer ('91, '93), and Hoelzl ('93)—the latter in a series of 60 cases from 1 year to 71 years of age—added several histological details, calling attention to the prominence the large cells of the theca (i.e., interstitial cells) may attain. H. Rabl ('98) made a careful study of atresia (25 cases) and supplemented it by comparative observations. He recognized the interstitial cells as "nothing more than hypertrophied stroma cells." Clarke, Boshagen, Pinto, Fränkel, Seitz, Fellner, Wallart and Schaeffer have given consistent descriptions of atresia folliculi and the interstitial cells of the human ovary. Wallart from an examination of 67 ovaries from fetal life (8 months) up to ninety-one years, concluded that the interstitial cells (termed by him the interstitial gland) are best developed and most closely massed during early years (up to puberty). During sexual maturity they are present but not so abundant, save during pregnancy. He recognized their origin from the theca interna of atretic follicles. A greater amount of atresia during the period of pregnancy and in the puerperium as indicated by the observations of Sinety, Wallart, Seitz, Pinto and Fellner, would be expected a priori. Whether or not the interstitial cells so formed should be regarded as an internal secretory gland having peculiar relations to pregnancy is of course another question. The more follicles reach maturity and rupture (forming corpora lutea) the less the degeneration, hence the appearance of balance and vicarious development that appealed to Aimé, and Ancel and Bouin. From my personal observation I may state the atretic follicles and their lipoid-containing theca cells (interstitial cells) are easily demonstrated in the human ovary by appropriate methods. The latter are more insignificant than in the cat, and doubtless relatively less persistent (as such) in the larger ovary. The

time the cells remain in the interstitial cell state, the amount and rapidity of the follicular degeneration and the size of the ovary, and possibly the type of 'metabolism' are evidently determining factors. At one extreme are such ovaries as the rabbit's, at the other those of mare or cow.

A brief discussion of the interstitial cells of the testis may be permissible even though no personal work has been done by me on their structure or origin. They appear to be quite abundantly present in the fetal testis (numerous papers), less developed during childhood, becoming numerous again at puberty and demonstrable in the active testis, thence forward during life. Their connective tissue origin appears to have been quite clearly shown. Their most striking histological feature is their lipoid and granular content. In histogenetic origin and structure they resemble closely the interstitial cells of the ovary, and like these are connective tissue (stromal) elements which for some causes have undergone the typical hypertrophy and lipoid change. In the testis, however, the analysis of the conditions and relations that underlie the appearance of these cells is far more difficult than in the ovary. In the latter organ the oogenetic growth processes are more distributed in time and place; in the testis, in close proximity are spermatocytes, spermatogonia, spermatids, maturing spermatozoa; and the indifferent or Sertoli cells—comparable to the ovarian follicle cells—are undergoing their progressive and regressive changes in association with the maturation of the male reproductive elements. In the anura, reptiles, birds and mammals this is the case. It is necessary therefore to turn for definite morphological clues to the lower forms, where the processes, progressive and regressive, occur in distinct portions of the organ. Despite the excellent studies of Allen, Whitehead and others I believe that there has not been made a full study of the development of the testis with a view to determining the growth correlations that lead to the appearance of the interstitial cells, and such an investigation might add much, even in the complex mammalian testis. That there is such a correlation between the tubule and interstitial cells would appear from the fact that the interstitial cells are not recorded as

existing by themselves—in the absence of seminal tubules, although tubule and interstitial cells seem to be often in reciprocal relation—atrophic changes in the tubule being accompanied by increase in the number of interstitial cells (Ancel and Bouin, Voinov; Biedl, p. 365). There is no evidence, therefore, that the factors that determine the transformation are intrinsic, that is, in the cells themselves. From the condition in the fetal testis, cryptorchid testis, the experimental results of vaso-ligation, and action of Roentgen rays (Biedl), it is clearly evident their presence is not directly correlated with the spermatogenetic process as such, but with the indifferent cells alone.

In fact, the absence of the reproductive cells—or the alteration in the processes in the indifferent cells correlated therewith, possibly of a distinctly 'degenerative' character—seems to particularly determine the appearance of these cells (fetal testis, cryptorchid testis, diseased condition).

Plato, in an excellent paper, attempted to correlate the presence of the interstitial cells in the reproductively active testis with the Sertoli cell and concluded that they fulfilled an essentially 'trophic function.' It may be mentioned, however, that during spermatogenesis the indifferent cells are continuously and alternately undergoing regressive as well as progressive changes, and that the largest part of the cytoplasm of the spermatids undergoes degeneration. Hence, it is rather difficult to determine with which phase of the cyclic activity of the Sertoli cells the correlation might be—the entire evidence seems to me to point to the regressive rather than the progressive. In the testis as in the ovary, therefore, it seems to me that the evidence of others indicates that the interstitial cell formation is related to processes that take place within the indifferent cells of the tubule, not directly correlated with spermatogenetic cells and in their absence, and of a fundamentally regressive character. With full cessation of activity within the tubule the interstitial cells would accordingly more or less completely disappear; as, it may be suggested, in extreme atrophy (Cushing's case xxxii, p. 277), and in hibernation (Tandler).

As to the quite distinct question—whether the interstitial cells of the testis, as such, form specific chemical substances which determine the development of the secondary sexual characters, or the appearance of sexual instincts—I only venture to suggest that since there is no direct evidence of their being such specific producers of ‘hormones’ of internal secretion, and since they never occur separately as far as known to me from the indifferent cells of the seminal tubules, considerable reluctance should be felt in deciding that they alone are the ‘gland’ that produces the substances with which the influence of the testis in the organism is correlated.

To Prof. S. H. Gage, the writer is indebted for numerous suggestions which are here gratefully acknowledged.

SUMMARY

1. The interstitial cells are modified stroma cells, and hence of connective tissue origin.
2. Their origin in the adult as an hypertrophy of theca cells during atresia folliculi is fully confirmed.
3. In the fetus, new born and immature kitten, they appear associated with the irregular so-called medullary cords and follicle formations of these periods.
4. Free lipoid granules appear in the indifferent cells of the atretic follicles, medullary cords and irregular medullary follicles in parts not associated with the ova.
5. The development of interstitial cells appears to be correlated with the activity of the indifferent or follicle cells in the absence of germ cells. The suggestion is strong that an element of degeneration is involved.
6. The zone in which they occur conforms to the centrifugal march of differential growth in the ovary.
7. No morphological value is believed to attach to the distinction of a fetal (or presexual) from an adult grouping of the cells.
8. No evidence is found for regarding the interstitial cells as constituting morphologically an intra-ovarian gland.

9. The recognition of them as constituting a physiological gland of ovarian secretion is regarded as without sufficient evidence.

10. A comparison with the interstitial cells of the testis is briefly presented. It is suggested that the same conditions determine their appearance in the testis as in the ovary.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Photograph of section of ovary, kitten 6 days P. P. Flemming's fluid fixation; no stain; no coverglass. $\times 30$. The groups of interstitial cells, appearing black from the contained fat, occupy a central position in the ovary about or close to the medullary cords. Two groups of few cells each occur in the stromal septa of the primitive cortex.
- 2 Photograph of ovary, kitten, about 5 weeks P. P. Flemming's fluid fixation; no stain; no coverglass. $\times 20$. Interstitial cell-groups appearing black. One small group in the primitive cortex.
- 3 Photograph of ovary, kitten, about 3 months P. P. Flemming's fluid fixation; no stain; no coverglass. Interstitial cell-groups black. It shows the relation of the interstitial cells to the irregular follicles of this period. Small groups of interstitial cells occur in the cortex. $\times 15$.
- 4 Photograph of ovary, cat, adult; Flemming's fluid; saffranin stain; interstitial cell-groups black.

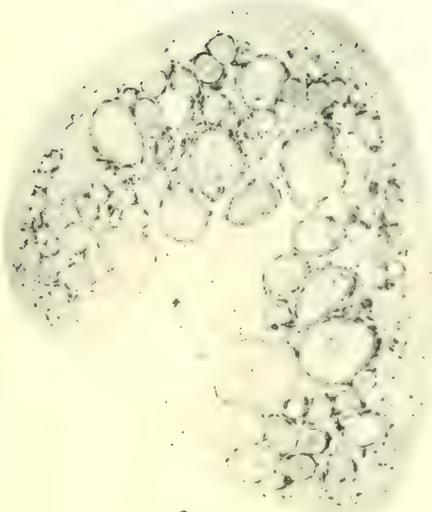
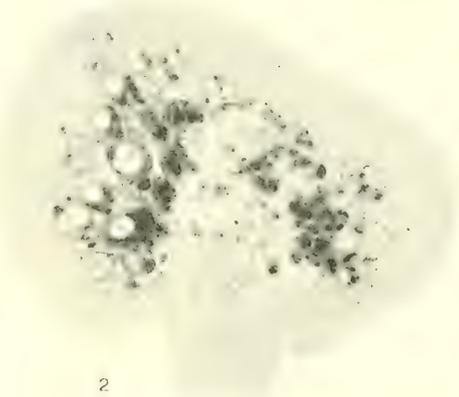


PLATE 2

EXPLANATION OF FIGURES

5, 6, 7, 8 Four stages of atresia folliculi, to show the origin of the interstitial cells from the theca interna. In the first figure the cells of the internal theca are hardly discernible. The shape and position of the ovum indicates beginning atresia. In the last figure the ovum has disappeared save the zona pellucida, and the antrum is nearly obliterated. Intermediate stages appear in figures 8 and 9. Photographs.

9 Photograph of section of ovary, young virgin adult; Hermann's fluid; no stain; interstitial cell-groups black. To show the typical picture of atresia, and its prevalence at this period. All the follicles save one are in some stage of atresia.

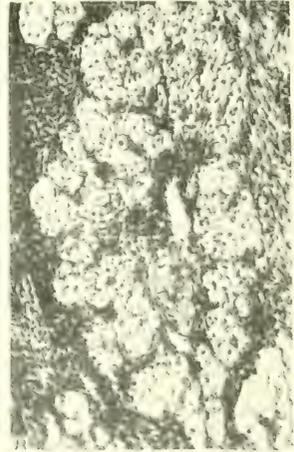
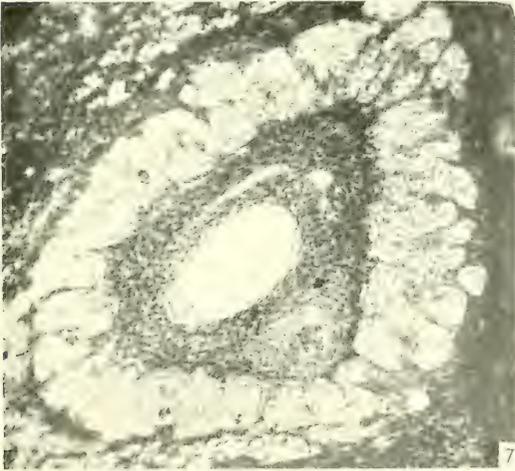
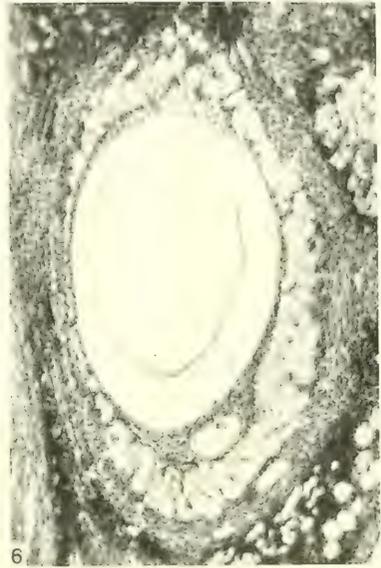


PLATE 3

EXPLANATION OF FIGURES

10 Photograph from a section of ovary of an adult cat; Flemming's fluid; saffranin. Four atretic follicles are shown.

11 Camera lucida drawing of a medullary cord; ovary of kitten 3 to 4 days P. P. Fat granules in the cells of the medullary cord are shown, as also the intimate relation of the interstitial cells to the parenchyma.

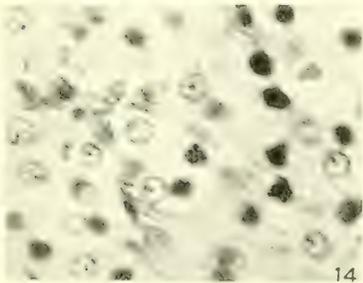
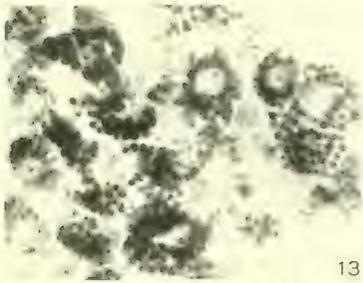
12 Camera lucida drawing of medullary cords; ovary of kitten, 3 to 4 days P. P.; two cords shown in transection.

13 Photograph of an interstitial cell-group from an adult ovary; cat; Flemming's fluid; saffranin; to illustrate the lipid content.

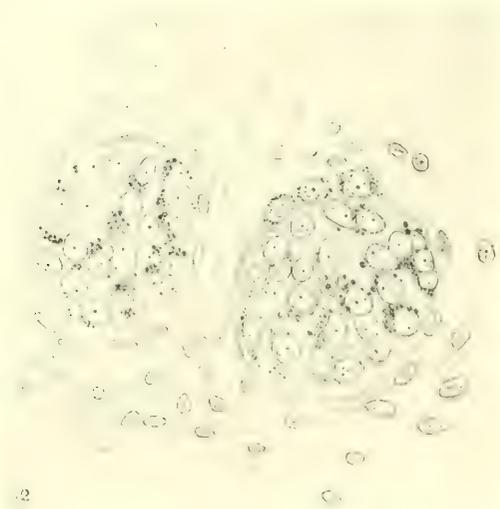
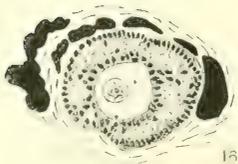
14 Photograph of an interstitial cell-group. Zenker's fluid; iron hematoxylin. The lipid content is therefore dissolved out; some of the nuclei are apparently pyknotic.

15 Drawing, showing the location of interstitial cell-groups in relation to the irregular medullary follicles. Kitten, about 5 to 6 weeks old. $\times 100$. The interstitial cell-groups are shown in black.

16 Drawing, as in figure 15.



11



12

THE TRACT OF LISSAUER AND THE SUBSTANTIA GELATINOSA ROLANDI

S. WALTER RANSON

The Anatomical Laboratory of the Northwestern University Medical School

ELEVEN FIGURES

There is located in the apex of the columna posterior between the substantia gelatinosa Rolandi and the periphery of the spinal cord an area of rather widely separated small medullated fibers. These fibers, having for the most part a vertical course, form a tract which is easily distinguished in Pal-Weigert preparations, in which it takes a lighter stain than the remainder of the substantia alba. It has for this reason usually been considered a part of the columna posterior, although it is admitted by all that it properly belongs with the longitudinal fiber tracts of the cord.

In 1885 Lissauer observed that fine medullated fibers grouped themselves on the lateral side of an entering rootlet, and, turning lateralward, separated themselves from the remainder of the radicle to enter the apex of the columna posterior. Here they turned to run vertically in the tract which now bears his name. These observations were confirmed by Bechterew, 1886. The general acceptance of these observations was facilitated by the results obtained shortly afterward by the application of the Golgi stain to the spinal cord. A number of observers, among them Kölliker ('91) working with foetal or newborn mammalian tissue, were able to show that the fibers of the entering dorsal root separate into two bundles, a lateral bundle of very fine axons and a medial bundle of much coarser ones. The lateral bundle of fine axons runs into Lissauer's tract, where the fibers divide into ascending and descending branches. The ascending limbs run upward in the tract for some distance, but the descending limbs are very short (Barker '99). This clear evidence in regard to

the axon content of the entering rootlets apparently established the correctness of the observations of Lissauer and Bechterew, and these have accordingly been generally accepted.

The usual account which is given of this tract is that it is formed of fine, rather sparsely arranged, medullated fibers, which enter the spinal cord from the dorsal roots. There is good reason to believe, however, that this account of the origin of these fine medullated fibers represents but half of the truth, since many of them seem to be of endogenous origin. The evidence in regard to this point has been presented in a previous paper (Ranson '13), and need be only briefly summarized here.

Nageotte ('03) was the first to assert that the medullated fibers of Lissauer's tract were of endogenous origin. He reported a case in which a tumor involved all the nerve roots in the cauda equina up to and including the fourth lumbar without causing any degeneration of the medullated fibers of Lissauer's tract. The presence of an apparently normal number of intact fibers in this case shows conclusively that many, probably a majority, of the medullated fibers of this tract are of endogenous origin. It can not be taken as conclusive proof that none of the medullated fibers in this tract are derived from the dorsal roots. Many human cords, in which extensive degeneration of the dorsal roots had resulted from tumors, syphilis and other causes, as well as the cords of animals in which some of the dorsal roots had been divided, have been studied with the object of tracing the degenerating fibers within the spinal cord. Most of these investigations, although showing extensive degeneration in the posterior funiculus, show no changes in the tract of Lissauer (see the papers of Darkschewitsch '96, Frölich '04, Kopezynski '06, Margulies '96, Orr '06, Wallenberg '98 and Zappert '98). A small amount of degeneration in Lissauer's tract after lesions of the dorsal roots has been seen by Collier and Buzzard '03), Laignel-Lavastine ('08), Sibelius ('05) and Sottas ('93).

The observations of Lissauer and those of Nageotte are diametrically opposed to each other. But, in view of the finding of a limited amount of degeneration in Lissauer's tract following lesions of the dorsal roots, it may be safely said that both are

in part correct and that the medullated fibers in this tract are in part endogenous and in part exogenous. It is probable that the endogenous fibers predominate.

In fact, the most recent work on this subject tends further to discredit the observations of Lissauer. Leszlényi ('12) made a comparative study of the tract of Lissauer and states that, while in man and many animals horizontal medullated fibers cross the tract to enter the substantia gelatinosa, the dorsal roots contribute practically nothing to the vertical fibers of the tract.

And, as we shall see, the observations of Kölliker and others on Golgi preparations are to be explained on another basis than that furnished by Lissauer's observations. In the cat it has been shown (Ranson '13) that the non-medullated fibers of the dorsal roots separate out from among the medullated fiber just before the rootlet enters the cord, and turning laterally they enter the tract of Lissauer. Most of the fibers of the lateral bundle of the dorsal root seen in Golgi preparations of the cord of newborn animals are fibers which never acquire a myelin sheath. The picture of an entering rootlet which is given by a pyridine-silver preparation of the spinal cord of an adult cat is very similar to that given by the Golgi method in the newborn animal, and very different from that seen in Pal-Weigert preparations of the adult cord.

We wish in this paper to present a study of the entering rootlets and tract of Lissauer in man, the rhesus monkey, the cat, rabbit, squirrel, guinea-pig, and albino rat, and to present at the same time some notes on the character of a closely associated structure—the substantia gelatinosa Rolandi.

TECHNIQUE

For the demonstration of the myelin sheaths sections were stained by the Pal-Weigert method. In differentiating these some sections were not fully decolorized in order to make sure that no fine medullated fibers were lost. The axons were stained by the pyridine-silver technique, the details of which were given in a previous paper (Ranson '11). In some cords, namely, those

of the rat and rabbit, we have succeeded in improving our preparations by a preliminary injection of ammoniated alcohol, as suggested by Huber ('13), but in other cords, especially those of the cat and monkey, excellent results can be secured without preliminary injection.

The pyridine-silver material was imbedded in paraffin and cut into sections 5 to 12 microns thick. The Pal-Weigert cellodin sections were 12 to 24 microns thick.

LISSAUER'S TRACT IN MAN

Parts of four apparently normal cords were obtained for me from autopsies on bodies which had been dead for two, three, six and twelve hours, respectively. The approximate levels of the sections were determined by reference to Marburg's Atlas, comparing the shape of the white and gray columns with that characteristic for each segment. In the third cervical segment (fig. 1) the gray substance proper, capped by the sharply pointed substantia gelatinosa, occupies about two-thirds of the columna posterior. The remaining one-third, the apex, is occupied by Lissauer's tract. In contrast to what is seen in the cat, the tract is not sharply limited either toward the fasciculus cuneatus or the lateral funiculus. It shows, however, no tendency to spread

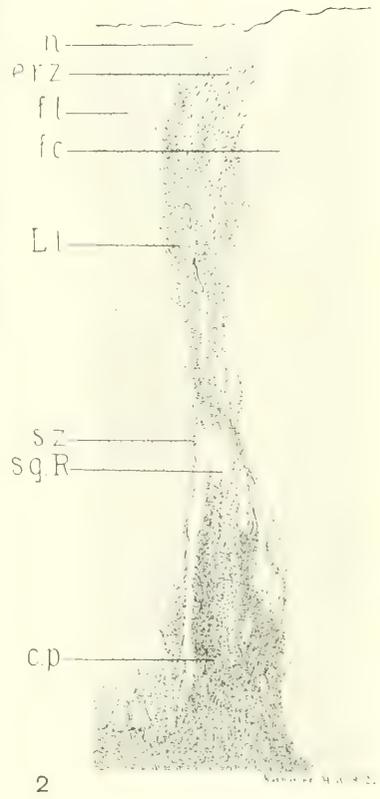
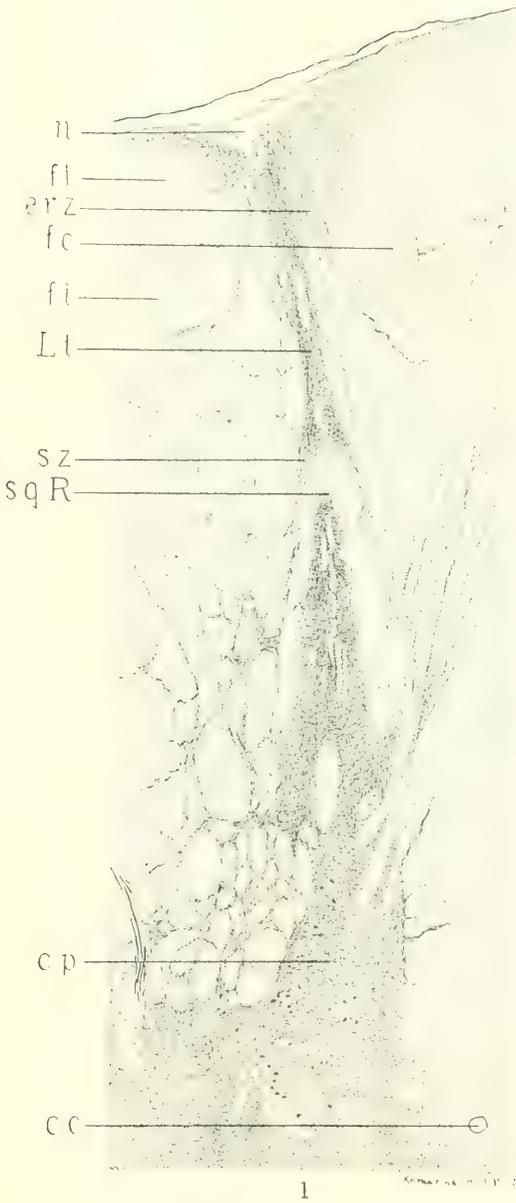
ABBREVIATIONS

The following is a list of abbreviations used on all the drawings:

<i>c.c.</i> , canalis centralis	<i>l.p.p.r.</i> , lateral part of posterior root
<i>c.p.</i> , columna posterior	<i>L.t.</i> , Lissauer's tract
<i>e.r.z.</i> , entering root zone	<i>n.</i> , neuroglia
<i>f.c.</i> , fasciculus cuneatus	<i>p.r.</i> , ring of pia constricting the entering root
<i>f.cs.</i> , fasciculus cerebellospinalis	<i>r.p.</i> , radix posterior
<i>f.l.</i> , funiculus lateralis	<i>s.g.R.</i> , substantia gelatinosa Rolandi
<i>i.l.</i> , intermediate layer	<i>s.z.</i> , stratum zonale
<i>l.e.L.t.</i> , lateral expansion of Lissauer's tract	

Fig. 1 From the third cervical segment of the spinal cord of man; pyridine-silver technique. $\times 32$.

Fig. 2 From the third thoracic segment of the spinal cord of man; pyridine-silver technique. $\times 32$.



downward along the lateral surface of the column posterior as in the monkey; but near the surface of the cord it becomes diffuse, spreading out into the fasciculus cuneatus on the one hand and the lateral funiculus on the other, and fading off gradually in either direction. The dorsal root fibers on entering the cord pass through the dorso-medial portion of the tract, cutting off the diffuse part of the tract in the fasciculus cuneatus.

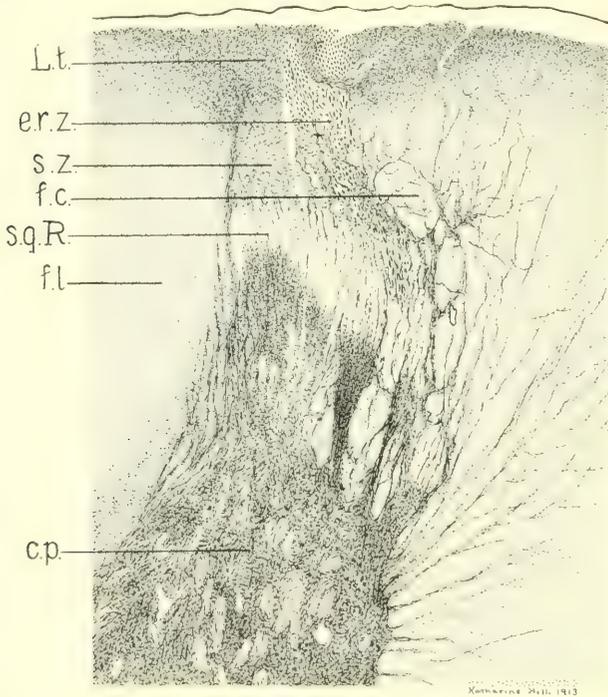


Fig. 3 From the third lumbar segment of the spinal cord of man; pyridine-silver technique. $\times 32$.

In the third thoracic segment (fig. 2) the apex represents about one-half of the length of the column posterior, and is filled by the tract of Lissauer which reaches from the substantia gelatinosa to the surface of the cord. It is not well-defined on either side, but does not spread out at the periphery of the cord as it does in the third cervical segment, nor does it extend forward

upon the lateral surface of the columna posterior as in the monkey. It is characteristic of the tract in the thoracic portion of the human cord that it is broken up by the entering rootlets, which run through it toward the gray substance.

In the third lumbar segment (fig. 3) the substantia gelatinosa reaches much nearer to the surface than in the two levels just described, and the tract of Lissauer is compressed between it and the surface of the cord. Instead of being long and slender, the tract at this level is short and broad. It is not sharply defined on either side, but spreads out near the surface of the cord into the lateral funiculus and even to a greater degree into the posterior funiculus. It does not extend forward upon the lateral surface of the columna posterior. The fila radicularia pass through it, breaking it up into two or more divisions.

Structure

In Pal-Weigert preparations the tract is clearly outlined from the other fiber columns by its light color. It contains fine and medium-sized medullated fibers rather sparsely arranged. These are for the most part vertical (in line with the long axis of the cord), but there are also horizontal and oblique fibers. In my preparations I was unable to see medullated fibers from the dorsal roots entering and becoming a part of the tract of Lissauer. Large bundles of medullated fibers run through the tract on their way to the fasciculus cuneatus and the columna posterior; but it is difficult to demonstrate individual fibers leaving these bundles to become a part of the tract. A good set of serial Pal-Weigert sections would probably show a few such fibers. Leszlényi believes that such dorsal root fibers as do enter the tract are horizontal and run directly into the substantia gelatinosa.

In pyridine-silver preparations the tract of Lissauer is stained much darker than any other part of the fiber columns of the cord and is seen to be composed of closely packed, fine axons, the majority of which are non-medullated. In the thoracic cord, because of the intimate relation of the entering radicles to the tract of Lissauer, it is more difficult to trace the non-med-

ullated fibers from the radicles into the tract than it is in either the cervical or lumbar regions. In serial sections of the third lumbar segment one can trace these fibers with ease. There is a tendency for these fibers to group themselves near the surface of the fila, they do not, however, form a compact layer on the surface of the fila, as in the cat. In other words, the non-medullated fibers are not entirely separated from among the medullated fibers of the rootlet until after the entrance of the rootlet into the cord. From the accumulation of non-medullated fibers near the surface of the entering radicle bundles of non-medullated fibers can be traced into the tract of Lissauer. The arrangement, while in all essentials the same, is by no means so clear and diagrammatic as in the cat.

LISSAUER'S TRACT IN THE MONKEY: MACACUS RHESUS

The spinal cord of the monkey is very favorable material for the pyridine-silver technique. The preparations are quite as good as those obtained from the cat, and considerably better than those which I have been able to secure of the human cord, or of that of the dog, pig, rabbit, squirrel, guinea-pig or rat. All of these cords give uniformly much poorer pictures than those of the monkey and cat.

In the cervical cord (fig. 4, seg. C. 7) the gray substance including the substantia gelatinosa occupies about four-fifths of the total length of the columna posterior, the apex with the included Lissauer's tract, one-fifth. The tract fills the apex and reaches from the substantia gelatinosa to the surface of the cord. An accumulation of subpial neuroglia is seen at its dorsal extremity (in the drawing the subpial neuroglia and the pia are together represented as a white band). The subpial layer of neuroglia, as well as the accumulation at the apex of the columna posterior and the septa which project into the substance of the cord, is granular not fibrous in appearance. This shows that neuroglia fibers are not differentiated and that there is no danger of confusing neuroglia fibers with fine axons. The limit of the tract is not sharp on the side toward the cuneate fasciculus; and there

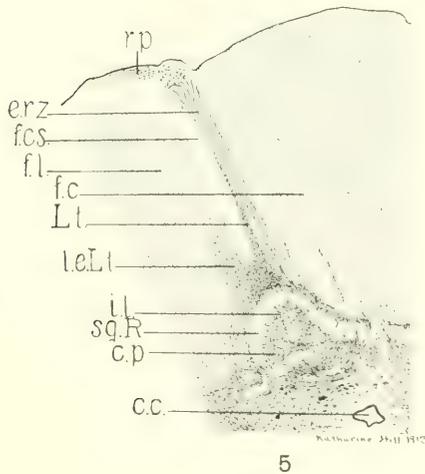
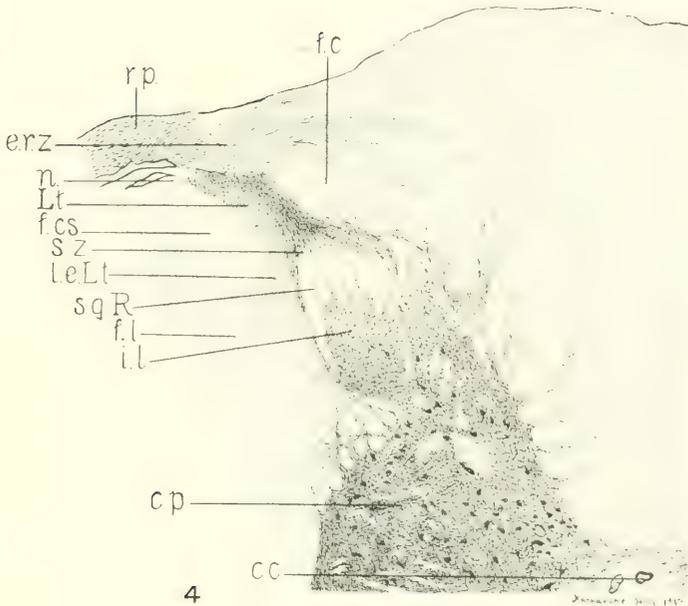


Fig. 4 From the seventh cervical segment of the spinal cord of the monkey (*Macacus rhesus*); pyridine-silver technique. $\times 32$.

Fig. 5 From the eighth thoracic segment of the spinal cord of the monkey (*Macacus rhesus*); pyridine-silver technique. $\times 32$.

is a considerable intermingling of the fibers of the two fascicles. On the side toward the lateral funiculus there is a neuroglia septum which extends into the cord, separating the tract in question from the cerebellospinal fasciculus. The septum does not, however, reach the gray substance, and ventrally to it the tract of Lissauer spreads out into the lateral funiculus upon the lateral surface of the column posterior. It goes over gradually into the fasciculus proprius of the lateral funiculus. The entering

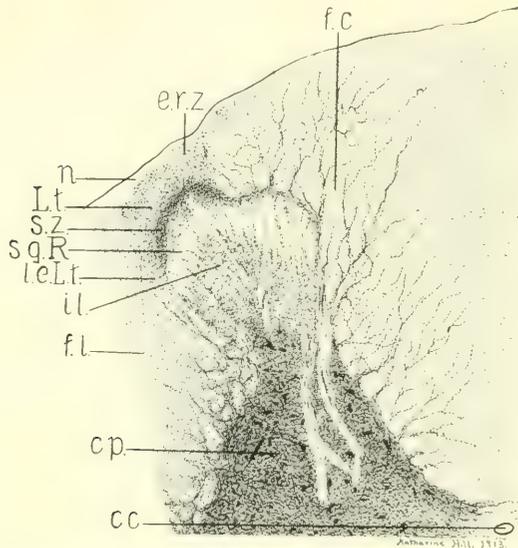


Fig. 6 From the fifth lumbar segment of the spinal cord of the monkey (*Macacus rhesus*); pyridine-silver technique. $\times 32$.

dorsal root bundles pass over the tip of the tract of Lissauer to enter the cord on its medial side. A few fibers are usually cut off from the main part of the tract by the entering radicles and form a small bundle in the dorso-lateral angle of the cuneate fasciculus.

In the thoracic region of the monkey cord (fig. 5, seg. T. 8.) the column posterior is very short, and is almost completely covered by the substantia gelatinosa. The main part of the tract of Lissauer extends dorsalward from the substantia gelatinosa as

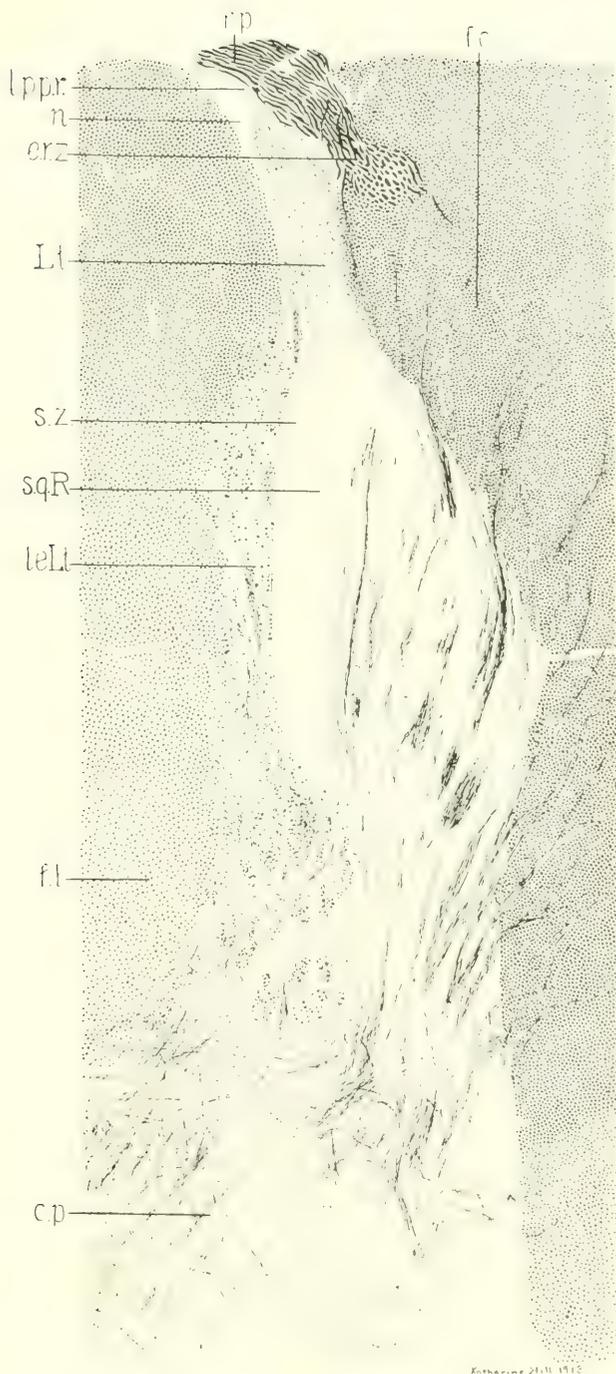


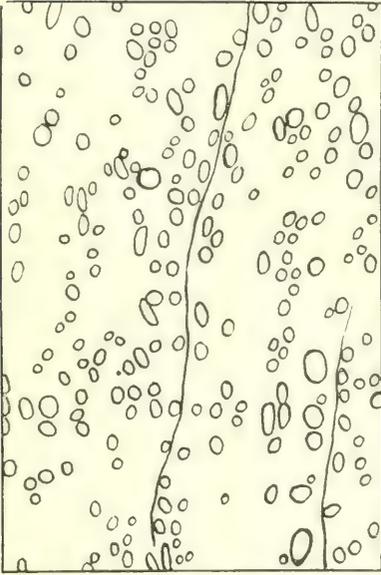
Fig. 7 From the fifth cervical segment of the spinal cord of the monkey (*Macacus rhesus*); Pal-Weigert technique. $\times 63$.

a sharply-pointed triangular area, reaching a little more than halfway to the surface of the cord. It is rather sharply demarcated from the posterior funiculus, but spreads out into the lateral funiculus, as indicated in the drawing. This lateral expansion also presents a somewhat triangular form with the apex pointing dorsally and separated from the apex of the main part of the tract by the cerebellospinal fasciculus. It fades out ventrally into the funiculus proprius on the lateral surface of the columna posterior. The entering dorsal root bundles run toward the posterior horn upon the medial side of the tract.

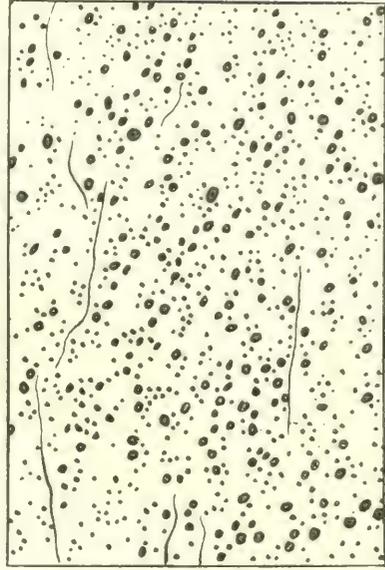
In the lumbar portions of the cord (fig. 6, seg. L. 5.) the columna posterior reaches nearly to the surface of the cord and the tract of Lissauer is compressed between it and the accumulation of subpial neuroglia in the apex. The tract caps only the lateral half of the substantia gelatinosa and is rather sharply delimited toward the medial side. It is less sharply limited on the lateral side and extends ventrally along the lateral surface of the columna posterior as in the cervical and thoracic segments. The entering radicles pass over the dorso-medial surface of the tract, and cut off from the main part of the tract a few fibers which form a small bundle near the surface of the fasciculus cuneatus.

Structure

The tract in the fifth cervical segment (figs. 7 and 8) contains a relatively small number of medullated fibers of varying size. Some are of medium caliber but the majority are very fine. They are more widely separated than in the other fiber tracts of the cord, and the tract as a whole is for this reason very lightly stained in Pal-Weigert preparations. These fibers are for the most part vertical in their course, appearing in the sections as blue rings. There are also scattered oblique and horizontal medullated fibers running through the tract in a dorso-ventral direction. In almost every section through an entering radicle one can trace a few fine medullated fibers (fig. 7, *l.p.p.r.*) out of the radicle and into the tract of Lissauer. Many of these fibers undoubtedly pass directly through the tract as the oblique or



8



9

Fig. 8 From the fifth cervical segment of the spinal cord of the monkey (*Macacus rhesus*); Pal-Weigert technique; a small area of the tract of Lissauer showing medullated fibers. $\times 1160$.

Fig. 9 From the seventh cervical segment of the spinal cord of the monkey (*Macacus rhesus*); pyridine-silver technique; a small area of the tract of Lissauer showing medullated and non-medullated axons. $\times 1160$.

horizontal fibers just mentioned. One fiber was followed from the root more than halfway to the substantia gelatinosa. It is probable that others of these medullated dorsal root fibers go to form vertical fibers in the tract.

In that part of the tract which extends ventrally along the lateral surface of the column posterior there are a few large medullated fibers; but the small and medium-sized ones are more widely scattered than in the dorsal portion of the tract so that this region takes an even lighter stain than the dorsal portion. There are in this lateral portion a very few oblique fibers, so few that they are practically negligible. On carefully looking through a number of preparations I was unable to see a single

oblique fiber passing from this lateral part into the dorsal part of the tract. Leszlényi has made much of this spreading out of the tract of Lissauer into the lateral funiculus. He saw in a great variety of animals fibers running into Lissauer's tract proper from this extension in the ground bundle of the lateral funiculus, and concluded that many of the fibers of the tract were derived in this way from the ground bundle. Leszlényi was working with Pal-Weigert preparations and was impressed with the similar light staining and open structure of the two regions, and he was led in this way to attempt to show a connection between the two. The similarity in Pal-Weigert preparations is, however, not nearly so striking as in pyridine-silver preparations where both are seen to be crowded with fine axons. Although I can find no evidence in support of Leszlényi's derivation of the dorsal part of the tract out of oblique fibers from the lateral, I do not doubt that the two regions are intimately associated, and are best described together under the head of Lissauer's tract.

In pyridine-silver preparations, the tract of Lissauer is closely packed with many very small and a few medium-sized axons (figs. 4 and 9). The very small axons are non-medullated. If one compares a pyridine-silver preparation (fig. 9) with a Pal-Weigert preparation (fig. 8), one sees that the number of axons is far in excess of the number of myelin sheaths, but that the latter are about as numerous as the medium-sized axons. These closely-packed, darkly-stained axons give the tract its characteristic dark appearance in pyridine-silver preparations. As in Pal-Weigert preparations, most of the fibers are vertical; but there are some that are horizontal and oblique. In the extension of the tract in the lateral funiculus the fine, non-medullated axons are also very numerous. As has been mentioned, a few large medullated fibers are found scattered through this lateral area; and these give this region a lighter appearance than the dorsal part, although between these large fibers the fine axons are as closely packed as in the dorsal portion of the tract.

There are fewer oblique or horizontal fibers in the lateral than in the dorsal part of the tract, and practically none running

ventro-laterally. In case the fibers on the lateral surface of the columna posterior were derived from the dorsal root, one would expect such oblique fibers, since the dorsal root fibers would have to run ventro-laterally in order to reach a position lateral to the columna posterior. The absence of such oblique fibers leads one to suspect that this lateral extension of the tract is of endogenous origin. If this assumption is correct, the non-medullated fibers of the tract of Lissauer are in part of endogenous and in part of exogenous origin. It is, of course, possible that the non-medullated fibers in the lateral part have a long course and are displaced ventro-laterally as they ascend, just as the fibers of the fasciculus gracilis are displaced medially. This, however, is highly improbable.

In the seventh cervical segment the fasciculus cuneatus contains large and small medullated axons, the fasciculus gracilis only medium-sized medullated axons; so that the two tracts are clearly differentiated in the sections (fig. 4). But both fascicles are very poor in non-medullated fibers. This was also found to be true in the cat, and indicates in a negative way that the non-medullated fibers of the dorsal roots become separated from the medullated at their entrance into the spinal cord and pursue a different course within it. The light stain of the cerebellospinal fasciculus is due to the fact that it is composed almost exclusively of large medullated fibers.

It is possible to trace both fine medullated and non-medullated fibers from the cervical dorsal root into Lissauer's tract. But the number of medullated fibers taking this course is relatively small (fig. 7, *l.p.p.r.*). In pyridine-silver preparations bundles containing very large numbers of non-medullated fibers can be seen leaving the peripheral part of an entering radicle and running into the tract of Lissauer. These have been traced in great detail in the paper on "Lissauer's tract in the cat," and numerous illustrations given. Here we will content ourselves with one illustration. The entering rootlet shown in figure 10 is surrounded by a constricting ring of pia, through the upper part of which near the upper surface of the rootlet the section was taken. This constricting band is seen at two points in the drawing

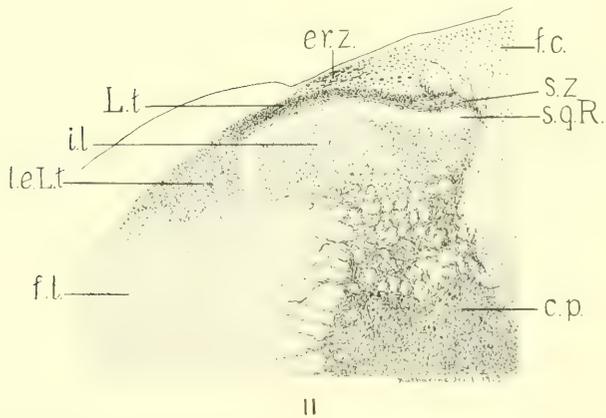
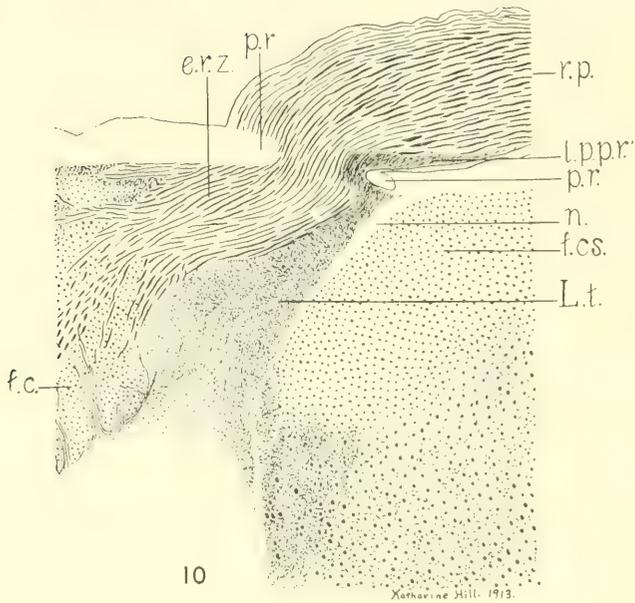


Fig. 10 From the seventh cervical segment of the spinal cord of the monkey (*Macacus rhesus*); pyridine-silver technique. $\times 63$.

Fig. 11 From the twelfth thoracic segment of the spinal cord of the albino rat; pyridine-silver technique. $\times 63$.

(each marked *p.r.*), and these two points are connected in the two preceding sections by the upper limit of the band. A study of the serial sections shows that the non-medullated fibers have

accumulated on the upper, lateral and lower surfaces of the rootlet. At *l.p.p.r.* in the illustration one sees a large bundle of non-medullated fibers from the layer on the lateral surface of the rootlet running over the constricting ring and into the tract of Lissauer. A study of the two sections just above shows that this layer of non-medullated fibers is continuous with that on the upper surface of the rootlet. These non-medullated fibers on the upper surface of the rootlet enter the cord between the constricting ring and the medullated fibers, and turning lateralward enter the tract of Lissauer. Followed downward in the series, a continuous layer of non-medullated fibers is seen passing over the lateral part of the constricting ring and entering the tract of Lissauer, just as in the figure given. On the under surface of the radicle one sees the fibers forming this part of the peripheral layer turning sharply lateralward underneath the entering medullated fibers to reach the tract of Lissauer.

It is very difficult to follow the course of the non-medullated fibers in the thoracic segments because of the close relation of the entering radicles to the tract of Lissauer. In the fifth lumbar segment one sees that as the rootlet passes over the apical accumulation of neuroglia to reach the medial side of the tract of Lissauer numerous small bundles of non-medullated fibers are given off which run ventrally through the apical neuroglia into this tract. It is this arrangement of the non-medullated fibers which was seen in Golgi preparations of newborn animals by Kölliker and others. The lateral part of the root, which they describe as consisting of very fine axons, is shown by pyridine-silver preparations to be still present in the adult and to take the same course into Lissauer's tract. The few fine medullated fibers which take the same course are by no means numerous enough to account for the lateral bundle of the dorsal root as seen in Golgi preparations. With the exception of a few small bundles of non-medullated fibers which are running toward the substantia gelatinosa, the entering root zone is free from non-medullated fibers—few, if any, enter the cuneate fasciculus.

The oblique and horizontal fibers of the tract pass forward into the substantia gelatinosa, and between this and the tract nu-

merous fibers are seen passing back and forth at all angles. The two structures are, in fact, most intimately associated and there is no sharp line of separation between them.

LISSAUER'S TRACT IN THE CAT

The tract of Lissauer in the cat has been described in detail in another paper. In shape and position it is very similar to the dorsal part of the tract in the monkey. It presents the same differences in shape and position in the three principal regions of the cord. In the cat it shows a tendency to spread out ventrolaterally, since the ventral part of the tract fades off gradually into the lateral funiculus. The chief difference in the two animals is, then, the great development of the lateral part of the tract in the monkey, of which there is only a suggestion in the cat.

The tract has the same structure as in the monkey. It is possible to follow a few medullated and a large number of non-medullated fibers into it from the dorsal roots. No oblique fibers either medullated or non-medullated could be followed into the tract from the ground bundle of the lateral funiculus. The substantia gelatinosa and the tract of Lissauer show the same interchange of fibers in the cat as in the monkey.

LISSAUER'S TRACT IN THE RABBIT

In the rodents the substantia gelatinosa Rolandi is highly developed, in some much more so than in others. Associated with the large and varying size of the substantia gelatinosa there are corresponding changes in the tract of Lissauer. There are more differences, so far as this tract is concerned, between the rabbit and the white rat than between the rabbit and man; and the study of the tract in the rodents is therefore of special interest.

Because of the similarity of the tract of Lissauer in the rabbit to that in higher mammals only a brief account need be given. In the seventh cervical segment the substantia gelatinosa is somewhat nearer the surface than in the same part of the monkey cord. The tract is about as long (antero-posteriorly) as

broad. It is more sharply defined medially than laterally, but not very sharply defined on either side. While the transition into the lateral funiculus is somewhat gradual, the tract can not properly be said to extend into that funiculus. The entering bundles from the dorsal root cut through the dorso-medial angle of the tract; and the small part which is thus cut off is spread out somewhat diffusely in the postero-lateral angle of the cuneate fasciculus. Bundles of non-medullated fibers can be traced into the tract of Lissauer from the dorsal roots. None of my preparations show medullated fibers from the dorsal roots entering the tract. But this may be due to the intimate relation of the entering root to the tract. As the bundles of root fibers pass through the tract some of them might separate from the others and become constituent fibers of the tract without being easily detected.

In the eighth thoracic segment the columna posterior is long; and the substantia gelatinosa is about the same distance from the surface as in the seventh cervical segment. But the space between the substantia gelatinosa and the surface of the cord is not fully occupied by the tract of Lissauer which lies close to the surface and is some distance from the substantia gelatinosa. The tract is very diffuse without sharp limits in any direction.

In the fifth lumbar segment the tract is located on the lateral extremity of the substantia gelatinosa and is short and broad. It is better defined medially than laterally; but the lateral extension which is well developed is outward along the surface of the cord rather than along the lateral border of the columna posterior. The entering rootlets pass through the dorso-medial angle of the tract, cutting off a small part, which lies as a thin band along the surface of the cord in the dorso-lateral angle of the cuneate fasciculus. Non-medullated fibers can be traced from the roots into the tract of Lissauer, but not in such a diagrammatic manner as in the cat and the monkey. I have not been able to trace medullated fibers from the dorsal root into the tract.

The structure of the tract is the same in the rabbit as in the monkey and the cat.

The tract of Lissauer in the squirrel is very similar to that in the rabbit and requires no special description.

THE TRACT OF LISSAUER IN THE ALBINO RAT

In the albino rat the substantia gelatinosa Rolandi is very massive, and differs in shape and position from that in the animals already studied. It does not vary much in shape from segment to segment, as in the other animals, but in the cervical, thoracic, and lumbar cord alike it reaches almost to the surface and is only slightly curved. In other words, it is spread out along the dorsal surface of the cord (fig. 11), from which it is separated by a very thin band of fibers representing the tract of Lissauer. The slight curvature brings the two extremities of the substantia gelatinosa somewhat farther from the surface. The superficial band of fibers has the same structure as the tract of Lissauer in other animals, consisting of scattered fine medullated fibers and closely-packed non-medullated ones. This band is very small in proportion to the size of the cord, and especially in proportion to the size of the substantia gelatinosa. At the lateral angle of the substantia gelatinosa the tract becomes continuous with an area which lies just lateral to the substantia gelatinosa and the caput of the columna posterior. This area in the lateral funiculus contains scattered small and medium-sized medullated fibers and is crowded with fine non-medullated axons. It has, in other words, the same structure as the lateral extension of the tract of Lissauer in the monkey. In the rat, due to the excessive development of the substantia gelatinosa, the tract of Lissauer proper has been reduced to a narrow strip between the substantia gelatinosa and the surface of the cord. The main bulk of the tract is located in the lateral funiculus in the region occupied in some other animals by a more or less diffuse lateral expansion of the tract. The tract of Lissauer and its lateral expansion are much the same in each of the three principal regions of the cord.

THE TRACT OF LISSAUER IN THE GUINEA-PIG

In the guinea-pig the tract of Lissauer is much like that in the rat. The substantia gelatinosa is excessively developed and occupies a position near the surface in each of the three principal regions of the cord. The tract is even less developed than in the rat, and is represented by a thin strip between the substantia gelatinosa and the surface of the cord. In the seventh cervical segment there is a lateral expansion of the tract similar to that in the rat, but less well defined. In the thoracic and lumbar regions the lateral expansion is less developed than in the cervical segments.

THE SUBSTANTIA GELATINOSA ROLANDI

The intimate and constant relation of the tract of Lissauer to the substantia gelatinosa and the interchange of fibers between the two suggests the possibility that the latter is the nucleus of reception of the afferent fibers of the former. This raises the question as to whether the structure of the substantia gelatinosa is in keeping with such a conception of its function.

According to all recent observers, nerve cells are very numerous in the substantia gelatinosa Rolandi. Ziehen ('99) has given a good summary of the literature on the nerve cells of this region. Rosenzweig ('05) and Sano ('09) have reworked the subject with the method of Bielschowsky. The larger cells of the zonal layer (the most superficial layer of the substantia gelatinosa) are well known. According to Cajal, their axons run into the lateral funiculus. But the most numerous and most characteristic cells are the small multipolar elements. These are very small, and, according to Rosenzweig, the substantia gelatinosa is 'crammed full' of them. All authors agree that they are demonstrated with difficulty because of the instability of their protoplasm, which is destroyed by the usual fixing and hardening agents. Sometimes the nuclei of these cells alone are visible in such preparations. In good Bielschowsky preparations (Rosenzweig) the substantia gelatinosa contains dendrites in large quantity derived

from these small cells as well as from the cells of the zonal layer and the cells of the nucleus of the columna posterior.

It is a fact of general knowledge that the substantia gelatinosa contains very few medullated fibers, with the exception of the bundles of fibers from the dorsal roots and the posterior funiculus, which run through it on their way to the more ventrally placed portion of the gray substance.

It has been known that non-medullated fibers were present in abundance and Rosenzweig ('05) and Sano ('09) have recently emphasized that fact. Contrary to the opinion of Weigert, Rosenzweig states that neuroglia cells and fibers are present in abundance. According to this author, nerve cells, axons, dendrites, glia cells and glia fibers fill the substantia gelatinosa completely. There is no intermediate gelatinous substance in a good Bielschowsky preparation, but this appears only in proportion as poor fixation has resulted in the destruction of the protoplasmic elements.

In pyridine-silver preparations the cells of the substantia gelatinosa are not very well stained and it is often impossible to distinguish between nerve cells and neuroglia. But the nerve fibers are well differentiated and it seems worth while to give an account of these as they are seen in the monkey cord, and to mention such differences as are seen in other animals.

The substantia gelatinosa presents two well-defined layers, the most superficial of which has received the name of stratum zonale. In the seventh cervical segment of the monkey cord this zonal layer (fig. 4, *S.Z.*) is especially marked in relation to the apex of the substantia gelatinosa, and projects as a triangular area into the tract of Lissauer. And from this apical mass the layer extends forward as a thin covering on either side of the substantia gelatinosa. In pyridine-silver preparations the stratum zonale is deeply stained because of the large number of fine axons which it contains. There is a constant interchange of fibers between it and the tract of Lissauer, and between it and the deeper layer. While the fibers run in every direction, there is a tendency for them to be tangential to the surface of the substantia gelatinosa. From the triangular mass at the apex numerous fibers run for-

ward through the thin part of the layer on either side of the substantia gelatinosa. There are also a very large number of vertical fibers. It would seem from its position and structure as if the stratum zonale served as a means for the passage of the fibers of the tract of Lissauer to and from the substantia gelatinosa. In Pal-Weigert preparations (fig. 7, *S.Z.*) this layer contains but very few fibers, most of which are horizontal and very few vertical in their course. It is easy to see that these are chiefly derived from the vertical and horizontal medullated fibers of Lissauer's tract, and the impression is also gained in Pal-Weigert preparations that this layer serves as a means of interchange of fibers between the tract of Lissauer and the substantia gelatinosa.

The second layer or substantia gelatinosa proper is somewhat lighter in pyridine-silver preparations and contains a considerable amount of light-yellow intermediate substance, which, as has been shown, the most recent authorities regard as an artifact due to the destruction of protoplasmic elements. The picture which this technique gives of this region is therefore not to be regarded as a complete one. This second layer contains, however, a great number of very fine axons running in every direction and forming a loose network. While the number of fibers is large, it is much less than in either the layer just dorsal or the layer just ventral to it, and for this reason it was represented rather light in the drawing to secure the proper contrast. The number of medullated fibers in this region is very small.

We should perhaps distinguish a third layer in the substantia gelatinosa, or better describe a layer of fibers just ventral to it and between it and the nucleus of the columna posterior. Here the fiber plexus is especially dense and there is as an added element a very large number of vertical fibers. In Pal-Weigert preparations only a relatively few medullated fibers are seen, and these are chiefly horizontal, running from the second layer of the substantia gelatinosa toward the nucleus of the columna posterior. Most of the fibers and especially of the vertical fibers in this region are non-medullated. Rosenzweig has called attention to this layer as a longitudinal conduction path com-

posed of non-medullated fibers and named it the 'Grenzschicht.' Kölliker ('91) has described it under the name of the "Plexus der Substantia gelatinosa." Ziehen ('99) regarded it as associated with the caput and not the substantia gelatinosa, and called it the "dorsalen Grenzplexus des Hinterhornkopfs." It seems probable that most of these vertical fibers are directly associated with the substantia gelatinosa.

In the thoracic region the stratum zonale contains more vertical fibers than in the cervical, and there is no sharp line of separation between it and the tract of Lissauer. It is clear that the fibers of the one pass directly over into the other. The vertical fibers of the 'Grenzschicht' or intermediate layer are especially well-developed in the thoracic region, and while the vast majority of them are non-medullated, there are a few medullated vertical fibers to be seen.

In the lumbar region there is an increase in thickness of the stratum zonale immediately underneath the tract of Lissauer: elsewhere it is represented by a thin layer. Here again it is clearly evident that it serves as a means of connection between the substantia gelatinosa and the tract of Lissauer. The vertical fibers of the intermediate layer are slightly more numerous than in the cervical cord, but not so closely packed as in the thoracic cord.

No mention has been made in this account of the bundles of fibers from the dorsal roots and the posterior funiculus, which pass through the substantia gelatinosa on their way to more ventral portions of the gray substance, since these cannot properly be considered as a part of its fiber complex.

The substantia gelatinosa in the cat shows the same close relation between the stratum zonale and Lissauer's tract. The intermediate layer of vertical fibers is the same in the cat as in the monkey and shows the same increase in number of fibers in the thoracic region. The same observations may be made on the rabbit. In the rat the vertical fibers of the intermediate layer tend to accumulate ventrally to the lateral angle of the substantia gelatinosa, especially in the cervical and thoracic segments.

SUMMARY

1. The tract of Lissauer is present in man and in the monkey, cat, rabbit, squirrel, rat and guinea-pig, and possesses the same structure in all.

2. It is composed of small, somewhat widely separated, medullated fibers, and great numbers of fine non-medullated axons.

3. While medullated fibers can be traced from the dorsal root into Lissauer's tract in some animals (cat and monkey), these fibers are not numerous. Some of these run horizontally through the tract to enter the substantia gelatinosa. It is probable that a few of them turn vertically in the tract to lose themselves among the other vertical medullated fibers of the tract.

4. It is clear that the number of medullated fibers entering the tract from the dorsal root is not sufficient to account for all the vertical medullated fibers found there, and that many, probably a majority of these vertical medullated fibers are of endogenous origin. This conclusion is in keeping with the results obtained from the study of the degeneration within the cord produced by lesions of the dorsal roots.

5. Great numbers of non-medullated fibers can be traced into the tract from the dorsal roots in man and in all the animals studied. These root fibers form a large part, perhaps the major part, of the non-medullated fibers of the tract.

6. In all cases there is manifested a tendency for the tract to spread out into the lateral funiculus at some level in the cord. This is particularly evident in the monkey and in the rat. Since there are no oblique fibers running ventro-laterally from the dorsal part of the tract into this lateral expansion of the tract, it seems quite certain that the fibers located here are not derived from the dorsal root. This leads to the conclusion that this lateral part of the tract is of endogenous origin. It would seem probable, therefore, that a part of the non-medullated fibers in the tract of the cat and other animals in which this lateral expansion is not so well developed are also of endogenous origin. Indeed, it would seem that the variations in the shape of the tract in different animals were chiefly due to variations in the position of the endogenous non-medullated fibers.

7. The narrow band-like tract in the rats and guinea-pigs covers a relatively small area. We can best explain the small size of the tract in these animals by assuming that most of the endogenous fibers are located in the lateral expansion of the tract in the lateral funiculus, and that the tract of Lissauer proper is in these animals chiefly composed of exogenous fibers. We need, however, more information on this subject.

8. The tract of Lissauer and the substantia gelatinosa Rolandi are intimately related to each other. But there seems to be no satisfactory explanation at present for the fact that in the rat and guinea-pig, where the substantia gelatinosa is excessively developed, the tract of Lissauer (exclusive of the lateral expansion) is least well developed. Sano's idea, that the enlarged substantia gelatinosa in a purely mechanical way pushes the tract of Lissauer lateralward, does not seem to be satisfactory. If this were the case, we would expect to find oblique fibers marking the course from the dorsal roots to the lateral expansion of Lissauer's tract.

9. Two layers can be distinguished in the substantia gelatinosa Rolandi and a third at the border between it and the ventrally-lying gray substance.

10. The most superficial layer of the substantia gelatinosa is the stratum zonale. It contains many non-medullated axons and fewer medullated fibers. It is most abundant immediately beneath the tract of Lissauer, and between the two there is a free interchange of fibers. From these facts one may assume that this layer serves as a means by which the fibers of Lissauer's tract reach the substantia gelatinosa.

11. The second layer, the substantia gelatinosa proper, contains a plexus somewhat less dense than that in the preceding layer. The fibers are almost all non-medullated.

12. The intermediate layer at the boundary between the substantia gelatinosa and the ventrally-lying gray matter is a dense plexus of fibers mostly non-medullated. A majority of these fibers have a vertical course. Rosenzweig has spoken of them as a special longitudinal conduction path formed of non-medullated fibers.

13. According to the observations of Rosenzweig and others, the substantia gelatinosa contains many nerve cells, mostly of small size, and a fine plexus of dendrites.

14. The tract of Lissauer, the intermediate layer of vertical fibers, and the substantia gelatinosa Rolandi are all intimately related to each other, and, taken together, form a complex system, the function of which is as yet unknown. Rosenzweig thinks that the substantia gelatinosa has a sensory function. Sano thinks that it may have an intimate relation to the sympathetic system, exerting a vasomotor and pilomotor control. He even suggests that it may be the locus of the cells of origin of efferent fibers in the dorsal roots. In view of the non-medullated character of most of the fibers belonging to this system, Sano's theory is especially interesting.

15. I believe we are justified in offering as a tentative interpretation the suggestion that we are dealing here with the apparatus for pain and temperature sensations. The non-medullated fibers of the dorsal roots have been shown to arise from the small cells of the spinal ganglion which are typical unipolar cells with T-shaped processes (Ranson '12). That is to say, from the location and form of the cells of origin of these fibers one may safely assume that they are afferent in function. They can have nothing to do with the afferent impulses which come from the muscles and joints, since these sensations travel upward in the fasciculus cuneatus and fasciculus gracilis, which contain practically none of these fibers. On the other hand, pain and temperature are known to pursue a different course in the cord from that taken by the muscle sense, passing into the gray matter near the level at which they enter the cord. This would correspond to the course of the non-medullated fibers since they are short and run in Lissauer's tract for only a short distance before entering the substantia gelatinosa. It is interesting to note that Ziehen has attributed to this part of the cord the function of conducting pain and temperature sensations. No data is at hand, however, as to how these sensations pass from one side of the cord to the other, although there is an abundance of fine commissural fibers to which this might pos-

sibly be assigned. It should be noted that the assumption that the tract of Lissauer and the substantia gelatinosa form an apparatus for the reception and conduction of pain and temperature sensations, does not exclude the possibility that we are dealing here with a center for vasomotor and pilomotor control, as suggested by Sano. In fact, these autonomic functions are of necessity closely correlated with the afferent impulses, which find their conscious expression in the form of sensations of pain, heat and cold. It is thus possible that the apparatus in question has a double function, serving at the same time as a central autonomic apparatus, and for the reception and conduction of pain and temperature sensations. We offer these suggestions in a most tentative way and without any attempt to support them by arguments, giving them rather as problems for future investigation than as conclusions to be drawn from the present paper.

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CONCERNING CERTAIN CYTOLOGICAL CHARACTERISTICS OF THE ERYTHROBLASTS IN THE PIG EMBRYO, AND THE ORIGIN OF NON-NUCLEATED ERYTHROCYTES BY A PROCESS OF CYTOPLASMIC CONSTRICTION

VICTOR E. EMMEL

The Department of Anatomy, Washington University Medical School

FIVE PLATES

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INTRODUCTION

The results presented in the present paper are concerned, first, with certain morphological changes in the structure of the erythroblast previous to the origin of the non-nucleated erythrocytes or plastids,¹ and second, with the mode of origin of the hemo-globin-containing plastids from these highly differentiated nucleated cells. Attention will be directed primarily to such observations which, so far as it has been ascertained from the literature of the subject, appear to contribute, in a degree at least, additional facts regarding the blood of the pig embryo, together with a discussion of the bearing of this data upon certain problems concerning the cytomorphosis of the mammalian red blood corpuscle.

The material consisted of 3 to 40 mm. pig embryos. The methods employed in the study of fresh and fixed blood vessels, and blood cultures will be indicated in the course of the ensuing description.

¹ The term 'erythrocyte' is here used in the sense adopted by Professor Minot ('13), in the Keibel-Mall Embryology "as a collective term for any and all red cells," while the term 'plastid' is employed to designate all non-nucleated red blood corpuscles.

CERTAIN CYTOLOGICAL CHARACTERISTICS OF THE ERYTHROBLASTS PREVIOUS TO THE FORMATION OF THE NON-NUCLEATED ERYTHROCYTES OR PLASTIDS

I. MORPHOLOGICAL CHANGES DURING CYTOMORPHOSIS

a. Form changes in the cell body

25 to 35 mm. pig embryos. Engel ('99), whose work represents perhaps the most extensive study, so far, of the blood of the young pig embryo, in describing the form of the typical large nucleated erythroblast (metrocytes II) of the 35 mm. pig, states that 'die Zellform ist kugelig' (p. 41). In the development of the nucleated red cells he distinguishes between the somewhat smaller erythroblasts with a larger nucleus and relatively smaller amount of cytoplasm which he designated as 'metrocyte I', and the more highly differentiated red blood cell larger in size, richer in hemoglobin, with a smaller, more compact nucleus, designated as 'metrocyte II.' Throughout the account, however, there is no indication that the typical normal form of these nucleated red cells is regarded as anything but rounded or spherical throughout their cytomorphosis, that is, up to the stage of their transformation into the non-nucleated forms.

The methods employed by Engel consisted in making cover glass preparations of the blood taken directly from the heart, liver, and other organs of the embryos as they were obtained fresh at the abattoir. After drying part of the preparations in the air and immersing the others in various fixing fluids, they were then transferred to his laboratory and stained. In the present investigation, the material first studied consisted of blood taken directly from the embryonic heart in the following manner: the pregnant uteri were either opened at once at the abattoir or conveyed to the laboratory, the transference in the latter case being made in a closed apparatus built on the principle of the fireless cooker, in which the material could be kept at the normal temperature of about 38°. After exposing the embryo the umbilical cord was clamped with a small serrefine artery clamp, the cord was then cut, the embryo removed from the uterus, and immediately placed in a warm chamber. The heart was then ex-

posed while still beating, grasped by its tip with a small forceps, severed from its vascular connections, and quickly brought into contact with the surface of a clean slide, the drop of blood thus deposited on the slide being disturbed as little as possible. A few of these preparations were allowed to dry in the air as in Engel's method, but as this procedure is open to criticism (Weidenreich '04, p. 373, and '11, p. 371) the greater part of the slides were inverted in a closed chamber and the blood cells fixed by exposure to the vapor of either 10 per cent formalin or 1 per cent osmic acid according to Weidenreich ('11), stained with Giemsa in the proportion of 2 drops of Giemsa to 1 cc. of distilled water, Maximow ('09, p. 457), with Erlich's triple stain, or Wright's stain. In addition to the fixed preparations, hanging drops of the fresh blood were studied microscopically in a warm chamber maintained at 38° to 40° temperature.

Confining our attention to the large nucleated erythroblasts, it may at once be stated that with regard to form the results of the first observations of these preparations seemed to substantiate Engel's account, for in the fixed as well as the fresh blood the appearance of the erythroblasts is such that, if no other methods be employed, one is readily lead to the conclusion that they are typically characterized by a rounded or spherical contour. However, a subsequent study of blood cells fixed *in situ* in the embryonic vessels of the foetal membranes yielded different results. These membranes, chorionic, allantoic, and amniotic, were fixed by immersion in Zenker formalin, that is, the usual Zenker mixture in which the 5 cc. of glacial acetic is replaced with 10 cc. of strong formalin, as recommended by Maximow ('09). These membranes were stained with Giemsa and mounted *in toto*. Upon examining the blood corpuscles thus fixed in the vessels it soon became evident that the erythroblasts were by no means all spherical in form. On the contrary, many of these nucleated cells were distinctly flattened biconcave discs, others bell shaped (figs. 3 and 4, *e*). These forms were so abundant it became evident either that previous conclusions were erroneous, or that the forms other than spherical were the results of faulty technique, although the latter seemed questionable since in all other respects the nuclei

and cytoplasmic fixation and stain of both nucleated and non-nucleated blood elements appeared excellent.

To decide this question it therefore became necessary to examine the fresh membranes directly and ascertain the form of the cells as they occur in the plasma while circulating through the living vessels. This was done at the abattoir. The embryos and membranes were removed from the uteri with the greatest possible economy of time, while the heart was still beating vigorously. A part of the foetal membrane was laid upon a slide on the stage of a microscope placed in a warm chamber, and the contents of the vessels at once examined. For this purpose the amniotic membranes proved the most favorable because of their transparency and ease of manipulation with the least disturbance to the contained vessels. It soon became evident that the majority of large nucleated erythroblasts in the vessels and capillaries of the membranes (of 25 to 35 mm. embryos) were typically nucleated biconcave discs, while others were more or less biconcave—convex or bell-shaped. In favorable regions where the circulation was sufficiently retarded² beautiful views of the blood corpuscles could be obtained as they passed a given point in the blood channel. This was especially true in one instance where an angle of a small vessel lay in the field of the microscope and the corpuscles could be followed as they passed around this angle in the vessel. The nature of the angle and the direction of the current were such that in the majority of cases the large nucleated erythroblasts almost in single file were regularly turned completely over before passing entirely around the angle. During one stage of the process of turning over, the erythroblasts almost invariably presented for a moment a biconcave or cupshaped profile. An instant later the same cell, upon presenting a flat view would assume the strikingly deceptive appearance of being spherical. Scores of erythroblasts were observed in this manner, always with similar results.

² It should be explained that in making these preparations the vessels had necessarily been severed in their connections with the heart so that the circulation referred to was in the nature of a streaming of the plasma in the vessel rather than a movement due to the beating of the heart.

It then remained to account for the spherical appearance of these cells as seen in slide preparations and hanging drops. If these were really more or less disc form, it should be possible to determine this point by turning the cells over so as to view all sides of a given cell. For this purpose a drop of fresh blood was placed on a carefully cleaned slide, covered with a clean rectangular cover glass, and the rim of the cover glass sealed with vaseline. The corners of the cover glasses used for this purpose had been previously heated and the tips bent down slightly, thus forming sufficient support to prevent pressure of the cover glass upon the blood cells. Upon studying such preparations it was found that by means of a very slight pressure produced with a pencil tip upon the somewhat flexible cover glass, in the vicinity of the microscopic field, the cells could be gently rolled about and turned over almost at will. Selecting large nucleated erythroblasts which appeared typically spherical in form, they were then manipulated in the above manner and it was discovered that in the majority of cases they could be turned so as to present a narrow profile which varied from an almost perfect biconcave disc to a more or less concave-convex or cup-shaped form (figs. 21, 41, 43 and 44). It was very difficult to keep these cells standing on edge for more than a moment on account of their constant tendency to turn over and lie flat upon the slide. This tendency in orientation accounts for the appearance of these cells as studied in dried or fixed cover-glass preparations, or even in hanging drops of fresh blood, for no sooner has the drop of blood been placed on the slide or cover-glass than the erythroblasts immediately orient themselves so as to lie flat upon the horizontal surface. In this position the appearance is very deceptive, for the flattened or disced contour in most cases can scarcely, if at all, be detected in the optical sections obtained by focussing, and consequently the observer may be readily led to the conclusion that they are spherical. It is interesting to note that Engel ('99) recognized a possible flattening of the younger erythroblasts (metarocytes I) in his dried preparations, but that he regarded this as the result of fixation and not a normal form is indicated in the following statement, made in connection with his observation that some of the erythroblasts

appeared somewhat larger in diameter after than before fixation: "Im Deckglastrock enpräparat erscheinen also die Zellen grösser als bei frischer Untersuchung, was wohl darin seinen Grund hat, dass im angetrockneten Präparate die kuglige Zelle durch die niedrige, angetrocknete Plasmaschicht flacher, dafür aber breiter wird" (page 39).

The results of these observations seem to leave no other alternative but the conclusion that the *typical normal form of the majority of the erythroblasts in the blood of the 25 mm.-35 mm. pig embryo, instead of being spherical, are biconcave-discoed and cup-shaped in form.*

Younger pig embryos. Younger embryos were next studied to ascertain transitional stages in the assumption of this form. The material consisted of both fresh and fixed blood, foetal membranes, serial celloidin and paraffine sections, fixation and staining methods being the same as in the previous preparations.

The youngest embryos obtained measured 3 mm. in greatest length. The primitive blood cells (mesamoeboids of Minot '12), and progenitors of the red blood cells as they lie free in the vessels of the embryo and the vascular network of the foetal membranes are uniformly spherical in form (fig. 1 *eu*). With Giemsa the cytoplasm takes a basophilic stain and as yet there is little if any evidence of the formation of hemoglobin. Younger blood cells in the blood islands of the yolk sac are more irregular in form, probably due in part to a not yet complete rounding up of the newly liberated primitive blood cells.

In 7 to 8 mm. embryos the early red blood cells are now becoming characterized by the presence of a small amount of hemoglobin, as indicated by the reddish tinge of the stained cytoplasm. In fresh preparations the living cytoplasm appears a very light yellow in color. While the majority of these red blood cells or primitive erythroblasts are more or less spherical in form, many of them now begin to show a definite biconvex lens shape (fig. 2, *ep*). These observations from serial sections and membranes mounted in toto were also verified in the study of fresh preparations, in which case the entire embryo with part of its membranes could be placed under a cover-glass upon the slide, sealed with

vaseline, and the blood cells examined in situ with the heart still beating. It may be mentioned that Maximow ('09) notes a similar modification in the shape of the erythroblast in the 13-day rabbit embryo and states that:

Die Form ist aber jetzt nicht immer regelmässig kugelig, sondern manchmal etwas unregelmässig und namentlich findet man sehr oft Formen, die mit dicken, stark bikonvexen Linsen verglichen werden können (fig. 4, Ebl rechts) und auch in dieser Beziehung ausserordentlich an die primitiven Erythroblasten des Hühnerembryo (Dantschakoff) erinnern. (p. 477).

The elliptical cells found by Erb ('65) in one-inch pig embryos appear also to correspond in form to the stage of cytomorphosis just described, for he states:

Im Blute zweier junger, ungefähr 1 Zoll langer Schweinsembryonen, die ich kurz nach dem Tode des Mutterthiers untersuchen konnte, fanden sich rothe Blutkörperchen von sehr auffallender Grösse und zum Theil von elliptischer Form, so dass sie bei oberflächlicher Betrachtung leicht für Froschblutkörperchen gehalten werden konnten; so hatten ohne Reagens die kreisunden rothen Blutkörperchen einen Durchmesser von 0,015-0,030 mm., die elliptischen einen langen Durchmesser von 0,035 mm. auf einen Kurzen von 0,020 mm. (p. 191).

In 10 to 12 mm. embryos the more highly differentiated erythroblasts contain more hemoglobin and are becoming characterized by a distinctly flattened disc form, while in 20 to 25 mm. embryos erythroblasts are now encountered which are approaching the form previously described for the 25 to 35 mm. embryos. Many of the cells have already assumed a well defined biconcave-disc shape (fig. 3, *e*), others tend toward a concave-convex or cup form. The hemoglobin content in these cells is well marked and approximates that of the mature erythroblast.

b. Correlated nuclear changes

The interesting subject of structural differentiation in erythrocytic nuclei will here be dealt with only to the extent of noting briefly certain morphological changes bearing on questions considered in the present paper. It is of course well known

that during cytomorphosis the erythrocytic nuclei undergo a reduction in size. This decrease in volume together with the associated increase in the density of the stain and compactness of the chromatin may be noted in figures 1, 2, 3, and 4. It will furthermore be observed in these figures that in the more fully differentiated cells the nuclei have come to occupy an eccentric position in the corpusele.

In addition to these well known nuclear changes there is an observation to be described concerning the form of the nucleus in the nature of mammalian erythroblasts which does not appear to have been previously noted, at least for the pig embryo. In the 3 mm. pig nuclei of the primitive erythroblasts are large round structures, containing scattered granules and masses of chromatin (fig. 1). It will also be observed that these large nuclei occupy the major part of the cell body. In embryos up to 10 mm. or even older, there is little evident deviation from the spherical form of the nuclei in the developing erythroblasts. On the other hand, in still older embryos—or what is more to the point, in the more highly differentiated corpuseles of young embryos—it appears that a departure from this form may occur. Although the nuclei as viewed in the mature erythroblast with the disc-like corpusele lying in a flat, horizontal position, as in the ordinary cover-glass preparations, may still appear round, if, however, the corpusele is rotated so as to present a profile view of the disc, a decided flattening of the nucleus may frequently be observed (figs. 3, 4, 6, 22 and 25). In other words, in conformity with the discing of the erythroblasts there is a tendency in at least the majority of the mature cells toward a correlated flattening of the nuclei. This flattening in nuclear form is evidently not to be confused with nuclear constrictions or fragmentation. The short axis of such a nucleus tends to lie in the same plane as that of the short axis of the disced corpusele. In some instances where the cell body has already approached the definitive form, while the nucleus is still more or less rounded, there may remain a slight cytoplasmic bulging or thickening of the rim of the disc in the region of the nucleus (fig. 41).

Concerning these results it may be observed that for the various types of tissue cells in the organism there is, with certain exceptions, a general relationship between the cell body and nucleus, such that the latter tends to conform to the former in shape. In view of this fact, this tendency toward a flattening of the erythrocytic nucleus in the pig embryo appears important as a further confirmation of the conclusion that the fully differentiated erythroblast normally tends to assume a biconcave disc or cup shape, for it is not readily evident how such a correlation in these two structures could be the result of any artificial or abnormal conditions of manipulation or fixation. It is not without interest also to note further that for lower vertebrates, in which the definitive erythrocytes are permanently nucleated, a similar flattening of erythrocytic nuclei in conformity with the lens or disc shaped cells has been recorded by Rawitz ('00), for certain ganoids and teleosts, by Giglio-Tos ('97), for amphibia, and by Pouchet ('79) for triton.

In conclusion, then, concerning changes in the form of the nucleated red blood corpuscles in 3 to 35 mm. pig embryos, it appears evident that *during their cytomorphosis the erythroblasts normally pass through a series of successive transitional changes from a spherical to a biconcave disc or even concave-convex bell form, accompanied by nuclear changes, which include a decrease in size, and assumption of a more or less eccentric position, and a tendency toward a flattening in conformity with the shape of the cell body.*

c. Discussion concerning the occurrence of similar changes in other mammals

In generally accepted accounts of the blood cells of the mammalian embryo, the normal form of the erythroblast is described as typically rounded or spherical. There is, however, some ground for questioning whether it is not necessary to determine to what extent this conclusion is to be regarded valid, for there is considerable evidence indicating the assumption of a disc or cup shaped by nucleated erythrocytes in a number of different animals.

In man for example, Weber ('46) who seems to have been the first to give a description of the nucleated red cells of a 12-weeks human embryo, concluded that these cells are spherical in form and describes them as "glätte kernhaltige Kügelchen von 0.0042" mittlerer Grösse." Also Engel in 1899, referring to human embryos of about 40 mm. draws a similar conclusion and states that "Die Metrocytes I und II Generation sind kugelige, selten ellipsoide Zellen, mit einem runden Kern, der bei den Metrocytes I Generation ein Drittel bis die Hälfte, bei denen II Generation ein Sechstel bis ein Achtel des Protoplasmaleibes beträgt" (p. 34). These statements are apparently accepted in even the recent literature on the human embryo as presenting an essentially correct account of the form of the erythroblast. On the other hand, it is important to note that soon after Weber's paper, Kölliker ('46) gave quite a different description of the erythroblasts of a 3-months human embryo. He stated on the contrary that "Die meisten Körperchen sind platt, wenige vertieft oder vollkommen kugelig, eine noch geringere Zahl derselben, und zwar im Leberblute, rundlich elliptisch" (p. 113), and concludes that "Die farbigen kernhaltigen Blutkörperchen platten sich je älter sie werden immer mehr ab, bekommen selbst leicht Excavationen" (p. 157). In the 2.5 cm. cat embryo, Howell ('91) describes finding red corpuscles which were "large, oval and nucleated, resembling somewhat the corpuscles of the reptile or amphibia. In shape they were biconcave, irregular, or apparently, in some cases, biconvex" (p. 59). It may be further noted that even in the lower vertebrates, where of course the erythrocytes are with few exceptions always nucleated, there is evidence according to Gage ('88) for *Petromyzon marinus*, and Dekhuyzen ('99, '01), for *Petromyzon fluviatilis*, that nucleated red cells may become biconcave and concavo-convex or bell shape (in the latter case designated 'chromokrateren' by Dekhuyzen); nor should it be overlooked that Dekhuyzen ('99) describes the occurrence of nucleated cup-shaped forms in the bone marrow of the rat, guinea-pig and rabbit, and draws the conclusion that "Bei der Autogenese der roten Blutkörperchen der Säugetiere tritt die ancestrale becherförmige rote Blutzelle als ein bald vorübergehendes Stadium auf" (p. 211).

2. CYTOPLASMIC PROCESSES

Not infrequently one encounters in the circulation of the pig embryo a red blood corpuscle which instead of having the usual smooth contour, is characterized by the presence of a cytoplasmic process projecting from its surface. Typically this process tapers to a somewhat blunt point (fig. 7). Occasionally the tip of the process may be partly bifurcated. A few cells were found having more than one process. These structures occur in the non-nucleated erythrocytes as well as in the erythroblasts (fig. 8). Since these processes are present in the fresh as well as in the most carefully fixed vessels, and the corpuscles having them appear otherwise perfectly normal in both their structure and stain, they are evidently to be regarded as structures normally present in the embryonic circulation. Engel ('99) also observed these cytoplasmic processes in fixed preparations of the blood of the pig embryo, and regarded them as normal structure:

Wie im frischen Zustande zeigten auch einige Metrocyten I Generation ein, selbst zwei spitz zulaufende Enden, von denen wohl nicht erst betont zu werden braucht, dass sie als Kunstproduct nicht anzusprechen sind. Eine Erklärung für diese Zellform muss ich mir so lange versagen, als es mir noch nicht gelungen ist, noch jüngere Schweineembryonen zu untersuchen. Es müsste sich dann zeigen, ob die durch ihre hämoglobinhaltigen Protoplasmafortsätze, breiten Spindelzellen ähnlichen Blutkörperchen, möglicherweise fixe, hämoglobinhaltige embryonale Endothelzellen gewesen sind, bevor sie die runde Form der freien Blutkörperchen erlangten (p. 39).

The question at once arises as to the origin of these structures. Engel's suggestion that they may represent remnants of the original endothelial cells, which had not yet disappeared in the course of differentiation, does not appear tenable for two reasons. First, these processes appear to occur more frequently in 25 to 35 mm. embryos than in younger specimens, and second, what appear to be identically similar structures may also be found in the non-nucleated corpuscles. An explanation which has suggested itself in the course of the present study is that the primary origin of these cytoplasmic structures is to be traced back to processes of cell division. As is well known, in the young mammalian embryo mitotic division of the young erythroblast may

take place in the blood even while actively circulating in the vessels. Figure 9 represents two instances of mitosis in circulating blood in which the two daughter cells are still connected by a strand of cytoplasm (cf. also fig. 42). It is readily seen that a final separation of this strand without an immediate rounding up of the newly formed cells would give rise to tapering, pointed cytoplasmic structures similar to those under consideration. Processes in the case of the non-nucleated plastids may also arise in a somewhat similar manner by cytoplasmic constrictions, for, as will be described later in the study of blood cell cultures, erythroblasts were observed to undergo a process of constriction into two parts, the one nucleated, the other non-nucleated and similar in appearance to a plastid; considerable evidence is at the same time also advanced for the normal occurrence of a similar phenomenon in the circulation. Occasionally plastids arising in this way were observed to constrict off in such a way as to leave a slender cytoplasmic process projecting from the surface in the region where the final separation from the parent erythroblast took place (figs. 20, 17, 13 and 32). The foregoing observations seem to offer an adequate explanation for all of these cytoplasmic structures with the exception of a few instances where a cell may have more than a single process or a single process is partly bifurcated, in which case possibly other factors may be involved.

Concerning the persistence of cytoplasmic strands during the division of the cytoplasm of the cell body, Jolly ('04) records the observation of a similar phenomenon in his study *in vitro* of the red blood cells of triton. In describing the division of these cells he states "il arrive enfin un moment où les deux cellules ne sont plus reliées que par un pont très mince et très court qui ne tarde pas à se rompre. Il peut se faire que ce pont persiste un certain temps, mais c'est absolument exceptionnel" (p. 621). In older cultures he found that sometimes these strands may be somewhat longer and thicker and persist for a longer time, in which case he infers that the cytoplasmic activity necessary for the complete separation has been interfered with. It is of interest, also, to note that in figure 17 of Jolly's ('07) monograph, which is given to illustrate nuclear extrusion in the bone-marrow from the white rat

examined *in vitro*, that the nucleus "reste longtemps accolé à l'espèce de cuticule qu'il vient de quitter" (p. 264). In mammals the occurrence in the circulation of erythrocytes with these cytoplasmic processes appears to be limited to the embryo. At least the writer is not at present aware of similar observations for the adult blood, except under certain pathological conditions as in cases of anemia. A possible explanation suggests itself for this fact is that in the embryo, in contrast to the adult, mitosis and cytoplasmic constriction in the erythroblasts takes place directly within the embryonic blood vessels. Here merely the passive movements of the blood corpuscles as they are whirled along in the blood current may be expected to interfere with the normal completion of the final stages of cytoplasmic separation or constriction in the dividing erythroblasts, especially in the later stages of their cytomorphosis, whereas in the adult these processes take place in a comparatively quiet environment, such as that of the red bone marrow.

3. ORIENTATION OF MATURE ERYTHROBLASTS WITH REFERENCE TO GRAVITY

Attention has already been called to the fact that the biconcave disc-shaped erythroblasts of the 25 to 35 mm. pig embryos tend to assume a flat position upon the horizontal surface on which they are resting, whether this surface be that of a glass slide or the wall of a blood vessel. A further interesting observation regarding the orientation of these erythroblasts which, so far as I have been able to ascertain, has not yet been elsewhere described, remains to be presented.

In mechanically turning the cells over by means of a gentle and repeated tapping on the cover-glass it was discovered that, in the case of the nucleated discs with the nucleus eccentric in position, the edge of the disc which rises first from the horizontal surface of the glass slide is the part opposite the nucleus (fig. 21, *a* and *b*). If the disturbance is not too vigorous and the cell is observed closely as it turns, this raised part of the rim of the disc will gradually approach the observer until only a profile view

of the cell body is presented (fig. 21, *c*), the turning is then completed and the cell again lies flat upon the slide but with the previously lower face of the disc now uppermost (fig. 21, *d*). In other words, during the entire process of rotation the side of the disc containing the nucleus tends to remain nearest the slide as if loaded, so that the cell body appears to rotate with the nucleated portion as a more or less fixed point, indicating that this part of the disc is the heavier portion of the erythroblast. This experiment was performed repeatedly with such uniform results that it was found almost impossible to obtain, for the purpose of making a camera lucida drawing, a satisfactory profile view of the cell with the nucleus uppermost. Similar observations were made on the living vessels where the erythroblasts, freely suspended in the plasma and not disturbed by adjacent cells, always tended to swing with the nuclear pole undermost. The same phenomenon is also most strikingly demonstrated in erythroblasts from which the hemoglobin has been removed by hemolysis. In this case also the nucleus remains suspended on the under side of the so-called shadow.

4. UNEQUAL REACTION OF CYTOPLASMIC AND NUCLEAR POLES TO OSMOTIC CHANGES

Figure 22 represents an erythroblast from the blood of a 28 mm. pig embryo kept in Ringer's solution for about forty-eight hours, and figure 45, a similar cell from a four-day autoplasmic culture of a 33 mm. pig embryo. These erythroblasts do not show any special evidence of degenerative change other than a swelling of the cell body due, presumably, to the absorption of a quantity of water, so that the previously disc-shaped corpuscle has assumed a rounded appearance. The important point here is that this reaction has not manifested itself uniformly throughout the whole cell body. This is not so evident when the cell is lying flat and undisturbed as in *a*; but when it is rotated so as to present a profile view, as in *b*, it will be observed that the swelling reaction has taken place largely, if not entirely, in that region of the disc opposite the nucleus, which may be conveniently designated as the

cytoplasmic pole in contrast to the region containing the nucleus or nuclear pole. In other words, in this reaction while the cytoplasmic pole of the disc has become smaller and rounded out, the nuclear pole has undergone but little modification. In some cases the contrast between these two poles is even further marked by a paler appearance in the fresh unfixed preparations, of the nuclear pole as compared with the opposite yellower hemoglobin containing cytoplasmic pole. Incidentally, it is a matter of further interest to note that the profile views of such cells may present appearances deceptively approximating one of nuclear extrusion which, as a precaution, should possibly be taken into account in evidence advanced for denucleation by a process of extrusion.

This striking difference in the behavior of the two poles of the erythroblast appears indicative of corresponding differences in morphological structure. It is generally recognized that the red blood corpuscle is composed of two parts, the one, consisting chiefly of a solution of hemoglobin and the other, the so-called stroma or colorless part. There has been considerable discussion as to whether, in the case of the non-nucleated erythrocyte, this stroma does or does not permeate the whole corpuscle as a cytoplasmic framework. One of the characteristic arguments advanced against the former of the two possibilities is based upon the changes in form which the biconcave disced corpuscles undergo when brought into contact with water, that is, into a hypertonic solution. Upon the absorption of water under such circumstances, the concave sides of the disc swell out and the corpuscles assume a spherical form. It is stated that,

This simple experiment conclusively shows that the corpuscle is composed of a membrane or external envelope with colored fluid contents. . . . On the other hand it is inexplicable on the supposition that the corpuscle is composed of a uniform disc-shape stroma permeated with colored substance, . . . for if this were the case, water should swell it out uniformly, as happens if a disc of gelatine is placed in water—the whole disc imbibing the water, and becoming increased in size while retaining its original shape (Schäfer '12, p. 369).

The extension of a similar argument to the reactions under consideration in the erythroblasts leads to the conclusion that there are important structural differences between the two poles of the

- fully differentiated erythroblast, a distinguishing feature of which would appear to consist in a predominance of a stroma structure at the nuclear pole, whereas the cytoplasmic pole of the disc, deficient in stroma, is composed of a greater amount of fluid or semifluid hemoglobin containing substance; (see also further discussion, pp. 147-148).

5. DISCUSSION WITH REFERENCE TO CERTAIN PROBLEMS IN THE CYTOMORPHOSIS OF THE MAMMALIAN ERYTHROCYTE

a. *The question of the correlation of the form of the definitive plastid with nuclear extrusion*

Concerning the origin of the biconcave disc form of the mammalian erythrocyte, Howell ('91) in a summation of the evidence for nuclear extrusion concludes that "the biconcavity of the red blood corpuscle is probably caused in the first place by the removal of the nucleus from the middle of the spherical cell" (p. 113). Regarding the same subject Maximow ('09) states for the rabbit embryo "Als Resultat dieses Kernaustrittes finden wir dann nackte, in dem Blutplasma frei flottierende Kerne und kernlose rote Blutkörperchen von mehr oder weniger regelmässiger Scheiben-oder Glockenform" (p. 485). Wiedenreich ('05, p. 445), in a discussion of the assumption of a cup form on the basis of the passage of a quantity of substance from the cell, notes "dass die Tatsache, dass die Entstehung der Napfform mit dem Kernverlust zusammenfällt, einen Beweis mehr für die Lehre vom Kernaustritt liefert;" (cf. also Weidenreich '10, p. 319).

These references are sufficient to make evident the tendency to explain the disc form of the definitive corpuscle as a result, in part at least, of the extrusion of the nucleus from the cell. In drawing this conclusion the erythroblast, previous to denucleation, is of course assumed to be spherical in form. But the observations considered in the present paper offer evidence that this assumption does not always hold, for it appears that in the pig embryo the erythroblasts may become disc or cup shaped while still retaining their nuclei. A possible explanation which has been suggested for these facts may be incidentally noted. Occasionally erythro-

blasts are found in which there has occurred a division of the nucleus into two parts, or a fragmentation into even a larger number of subdivisions. It has been assumed that a subsequent expulsion of only a part of the nucleus at a given time might thus result in a partial discing or cupping of the erythroblast, though the cell may still retain a nuclear remainder. Such an explanation, at least for the pig embryo, does not appear tenable for the following reasons. First, no evidence was noted indicating that the nuclei in the typical disc or cup-shaped cells were only remnants of previously divided and partially extruded nuclei, and second, it has been possible in the pig embryo to find occasionally such double nucleated erythroblasts, and they too, it is to be emphasized, had already assumed disc or cup forms as is illustrated in figures 12 *a*, 23 and 24. It seems clearly evident, then, that the assumption of the cell shape under consideration is by no means necessarily correlated with an extrusion of the nucleus. Weidenreich ('03 *a*), in a discussion of Dekhuyzen's observation of bell-shaped corpuscles in *Petromyzon fluviatilis* indicates an appreciation of this possibility in the remark "interessant wäre dabei jedenfalls, dass auch kernhaltige rote Blutkörperchen gäbe, die die Glockenform aufweisen, möglicherweise sie also keine Folge des kernverlustes zu sein braucht; selbstverständlich bedarf dies aber noch besonderer Untersuchung" (p. 475).

While the present facts, therefore, indicate that the assumption of a disc or cup form by the mammalian red blood corpuscle is not necessarily initiated by nor dependent upon the extrusion of the nucleus, the question may be considered whether there are any other factors which may account for the phenomenon. There is a substantial and growing body of facts not only for the conclusion that the mature erythrocyte is surrounded by a membrane (Weidenreich '05, p. 444, 445 and '11, p. 27) but also that this membrane is composed in part at least of lipoid substances such as lecithin and cholesterin. For example, Peskind ('03), after a study of the action of acids and acid salts on blood corpuscles, concludes that "theoretical considerations, as well as chemical and histological facts brought out in this paper, render the existence of an envelope in the mammalian corpuscle highly prob-

able, if not absolutely certain. To explain all the facts such an envelope must be assumed to contain nucleoproteid, cholesterol and lecithin" (p. 429); (see also Schäfer '12). Albrecht ('03), among the more recent investigators of the subject, has shown that globules of this myelin substance have a tendency to become thin at the center and thicker at the rim in such a manner as to force the fluid content to assume a disc form. One can readily verify this by making an emulsion of a quantity of lecithin in water or in a normal salt solution. Upon a microscopical examination of a slide preparation of such a mixture, the globules of lecithin can readily be seen becoming modified into flattened, biconcave disc, and even approximately cup-shaped forms. The experiments may be even more strikingly performed, as suggested to me by Dr. Bloor of the Department of Physiological Chemistry, of the Washington University Medical School, by placing a small quantity of lecithin upon a glass slide, adding a drop of water, covering with a cover-glass and examining at once. Evidently, then, the erythrocytic membrane includes elements which may play an important rôle in the tendency toward the formation of a biconcave disc or cup shape. Weidenreich ('03, p. 478), has also shown that this tendency may be evident even in the so-called shadows remaining after the loss of the hemoglobin from the corpuscles through hemolysis.

Returning to our original subject, on the basis of an intimate correlation between nuclear extrusion and the definitive form of the corpuscle, it would appear necessary to assume that the cell membrane plus its lipid elements is either not present or else does not become effective in the production of the definitive form until after the extrusion of the nucleus. Indeed Albrecht ('03), apparently appreciating this situation, has suggested that the myelin substance is actually added to the surface of the cell at the time of nuclear extrusion, for he states "es erscheint nicht unwahrscheinlich dass gerade bei und nach der Ausstossung des Kernes fettartige Substanz in grösser Menge an die Oberfläche der Zelle ausfliesst; dadurch erklärt sich nach Obigem der angegebene Uebergang in die Glocken- und Dellenform nach der Kernausstossung" (p. 19). But in contrast to this it may be pointed

out that since it has been shown that the cell may approximate the definitive form while still nucleated, it follows that an active lipid membrane may already be present in the nucleated erythroblast. In view of these facts the conclusion presents itself that the differentiation of an effective lipid membrane is not a matter of sudden formation, as intimated by Albrecht, but rather that it is gradually differentiated along with the formation of hemoglobin during the cytomorphosis of the cell, and that under certain conditions, as in the case of the large erythroblasts of the pig embryo, this differentiation may manifest itself by the gradual modification of the form of the erythroblast in the direction of a biconcave disc or cup shape even before the cell has given rise to a non-nucleated plastid. It is of course not to be overlooked that other factors such as the loss of water (Malassez '82, p. 22), an increase in the concentration of the blood plasma (Kölliker '46, p. 142), and variations in chemical composition such as the quantity of CO_2 (Hamburger '97, '02), may participate in the process. In connection with this conclusion of the gradual formation of a form determining membrane during cytomorphosis, it is of interest to note the conclusion of Minot ('12), that the "cell membranes probably developed at the same time as the hemoglobin" (p. 505), and that of Weidenreich ('05), that in the differentiation of the young, nucleated erythrocyte "die Membran durch eine einfache Ablagerung des Lezithins und Cholestearins, die ihre wesentlichen Bestandteile sind, in der äussersten Peripherie der homogenen Blutzelle zustande Kommt" (p. 444).

b. Possible factors accounting for the eccentric position of the nucleus in the erythroblast

Several explanations have been advanced for the change in position of the erythrocytic nucleus from the center toward the periphery of the cell during differentiation. Weidenreich ('05, p. 434), suggests that with the liquefaction of the hemoglobin containing cytoplasm, the nucleus may become relatively lighter in specific gravity and, being free, float toward the surface of the corpuscle. Jolly ('07, p. 273), while recognizing the possibility

of such a change in the weight of the nucleus, emphasizes an increase in hydrostatic pressure as a factor in both the assumption of an eccentric position as well as in the ensuing extrusion of the nucleus. The question still seems an open one concerning which some of the observations in the present study appear to justify a further discussion.

Concerning the explanation of this eccentricity on the basis of the nucleus lying in a fluid or semifluid medium, no evidence has so far been attained for the pig embryo, that the nucleus of the mature erythroblast is thus free inside the cell, nor was it ever observed to change its position during any mechanical manipulation of the blood corpuscles. On the other hand, some observations were made indicating a more or less fluid condition of the hemoglobin containing cytoplasm as illustrated in figure 35, where, in certain cells being studied *in vitro*, occasionally what appeared to be a vacuole was observed to move about in the cytoplasm. In the case shown in the figure there could be no question that the vacuole was located within the cell, as was determined by a mechanical rotation of the cell, nor could there be any doubt but that the vacuole actually changed its relative position for the corpuscle itself appeared to remain stationary. Concerning the assumed decrease in the specific gravity of the nucleus, the preceding observations regarding orientation in which the nuclear pole tended to swing undermost, while not conclusive, would if anything suggest a greater rather than a lesser weight for the nucleus as compared with an equal volume of hemoglobin containing cytoplasm.

On the other hand, some of the present observations are of a more positive character with reference to this question and have an interesting bearing on the problem. It will be recalled that in the previous description of the behavior of the cells in changed osmotic conditions that the reactions were of such a character as to indicate structural differences between the nuclear and cytoplasmic poles of the mature erythroblast. Such a structural difference appears further indicated by the occurrence in the circulation of the pig embryo (*cf.* p. 171) of so-called free erythrocytic nuclei surrounded by a rim of cytoplasm (figs. 10, 11 and 12),

in which case the nucleus which has become separated from the remainder of the cell in the formation of the non-nucleated erythrocyte or plastid, instead of being free is still surrounded by an appreciable quantity of adherent cytoplasm. These results suggest the conclusion that just as in the case of the differentiation of the lipid containing membranes, so here too, the assumption of an eccentric position by the nucleus is a gradual one correlated with the formation of hemoglobin. That some of the processes involved may in a manner be comparable to activities in secretory cells, in which the new product tends to accumulate in one part of the cell while the nucleus and part of the original cytoplasm is crowded toward the opposite pole of the cell. That the hemoglobin differentiates presumably in a fluid or semifluid form, while the lipid substances of the cell accumulate at the surface of the corpuscle, with the result that the remaining elements of the original cytoplasm, which have not become involved in the formation of hemoglobin, together with the nucleus have thus come to occupy the periphery of the erythroblast in a manner possibly not unlike the conditions obtaining with regard to the eccentric position of the nuclei in certain active gland cells, or as not infrequently occurs in various leucocytes at the height of their specialized differentiation. It remains to be seen to what extent this conclusion will be substantiated as the result of further investigation.

THE ORIGIN OF NON-NUCLEATED ERYTHROCYTES OR PLASTIDS

1. STRUCTURAL CHARACTERISTICS OF THE NON-NUCLEATED ERYTHROCYTES IN 10 TO 35 MM. PIG EMBRYOS

The red blood elements of the 10 mm. pig are almost all nucleated cells, although an occasional non-nucleated erythrocyte may already be present. With the further growth of the embryo from 10 to 25 mm. these non-nucleated erythrocytes gradually increase in number. A little later, at various stages between 25 and 35 mm., this increase is greatly accelerated, so that in embryos larger than 35 mm. or 40 mm., the blood, instead of consisting chiefly of nucleated red cells is now composed largely,

and in still later stages almost entirely, of non-nucleated hemoglobin elements or erythrocytes.

Confining our attention to these non-nucleated elements or plastids, as they occur in embryos between 10 and 35 mm., it will be observed that they vary greatly in size, ranging from the average sized plastids (fig. 29, 30 and 4 μm) to the large so-called macrocytes on the one hand (figs. 4 pl and 31), to microcytes, on the other hand, some of which are even comparable to blood platelets in size (figs. 4 ps , 11 ps , 26 and 27). The presence of the latter smaller plastids is in interesting contrast to adult mammalian blood where, as is frequently stated, that hemoglobin containing bodies the size of blood platelets do not occur in normal blood. But that even these smaller plastids in the early pig embryo in the majority of cases are hemoglobin containing elements is unquestionably indicated by their yellowish color in fresh preparations and their characteristic hemoglobin stain with Giemsa (figs. 4 and 11, ps). Engel gives no account of these smaller hemoglobin corpuscles, but their frequent and constant occurrence in both fixed and fresh blood, in the latter case, examined while still within the living vessels, seems to leave no doubt but that they are to be regarded as normal elements in the blood of the pig embryo at this stage of development.

In form these non-nucleated corpuscles vary from rounded or spherical structures (figs. 27 and 28) to biconcave discs (fig. 31) and concavo-convex or cup-shaped elements (figs. 29, 30 and 4). The spherical form appears to be more characteristic of the smaller plastids, although even some of these can be demonstrated to have a disc shape (fig. 26). While some of the medium sized and larger plastids may be spherical (fig. 28), the majority are either biconcave discs or bell shaped the latter form being apparently predominant (fig. 4). Engel ('99) recognizes a variation in size of these non-nucleated erythrocytes, but describes them all as spherical in shape, stating that in the 35 mm. pig embryos "Dellen sind meistens nicht nachweisbar" (p. 41), and that in the 40 mm. pig "Trotz der grossten Sorgfalt, welche ich auf die Anfertigung der Präparate verwenden konnte, war es meistens nicht möglich Präparate mit kreisrunden dellenhaltigen Blutscheiben zu erlangen" (p. 45), a conclusion which it seems

must be due primarily to the fact that the evidence was derived from dried cover-glass preparations, for if one studies the fresh blood either in the vessels of the membranes or on a slide under a cover-glass sealed with vaseline where the plastids may be turned over with proper manipulation it can be readily observed that whereas some of these plastids are spherical, others are unquestionably biconcave-disc or cup shaped. A few of the smaller elements may be elongated and oval or even more or less rod shaped. While the majority of the plastids have a smooth contour an occasional one is found possessing a tapering cytoplasmic process projecting from its surface (fig. 8; cf. pp. 138, 160, 170).

2. THE ORIGIN OF NON-NUCLEATED RED CORPUSCLES IN BLOOD CULTURES

Up to this stage of the present study attention has been directed to cytological characteristics of the large nucleated red cells or erythroblasts and the non-nucleated red plastids or erythrocytes. This brings us to the long standing question as to the origin of the non-nucleated elements, whether they arise from the nucleus, by an intracellular nuclear disintegration and dissolution, or by some other process. Though many facts and much argument have been advanced for various views, it does not appear that the question can yet be regarded as settled.

The usual methods of study employed in approaching the problem have consisted in the study of blood slides and sections of fixed and stained vascular tissues. The diversity of opinion, however, which still exists even after studies of this character, suggests the desirability of other methods of attacking the problem. The recently developed technique of tissue culture, with the striking results obtained by Harrison ('07, '10), Burrows ('10, '11), Carrel and Burrows ('11), Lambert and Hanes ('13), Lewis ('11, '12), Loeb ('12), Oppel ('12) and others, lead one to expect that similar methods may yield important data concerning the complicated problem of the life history and genetic relationship of the various types of blood cells—especially so when it is considered that Jolly ('11) succeeded in keeping amphibian leucocytes alive outside the organism for nearly a year. With this hope, a study

of the behavior of blood corpuscles in plasma cultures was begun somewhat over two years ago, some of the results of which, in so far as they relate to the question of the origin of the non-nucleated erythrocytes in the early pig embryo, will be here presented.

a. Material and methods

The material studied consisted chiefly of pig embryos from 25 to 35 mm. in length. At this time there occurs within the embryonic circulation a marked increase in the number of non-nucleated erythrocytes, so that whereas the blood of younger embryos consists almost entirely of nucleated corpuscles, the non-nucleated elements predominate in older specimens. Consequently, pig embryos approximating 30 mm. in length, might be expected to prove especially favorable for the investigation of the processes involved in the origin of the non-nucleated erythrocytes.

Through the kindness of the Krey Packing Company of St. Louis these embryos could be obtained under very favorable circumstances, so that the embryos could be removed from the uteri and the blood obtained within a few minutes after the killing of the parent animal. For some purposes the material was at once placed in a warm chamber set up at the abattoir. Within this chamber, maintained at 38 to 40°C., preparations of the blood as well as of the foetal membranes and their vessels could be immediately examined microscopically. More permanent cultures necessitated an incubator. For this purpose the pregnant uteri were removed to the Anatomical Laboratory of the Medical School. An apparatus based on the principle of the fireless cooker rendered it possible to make this transfer of the fresh material with little deviation from the normal body temperature, which in a preliminary examination of fifteen uteri just taken from the slaughtered animals, had been ascertained to range from 37 to 40°. A self-registering maximum-minimum thermometer was always carried with the specimens. The entire time elapsing between the taking of the uteri and their arrival at the laboratory occupied from thirty to forty minutes. Only embryos in apparently perfectly normal condition, with the heart still beating, were used for culture purposes.

The technique employed in making the culture was essentially that developed by Harrison, Burrows, and Carrel. Some homoplastic cultures were made by placing the blood cells of younger embryos in the centrifuged plasma of much older specimens. The best results for the present purpose, however, were obtained from autoplasmic cultures and the data to be described is chiefly from the latter kind of preparations. The fact that in the autoplasmic cultures from these young embryos the culture media does not coagulate appears one of great importance as furnishing the more favorable conditions for the continued normal life and function of the erythrocytes, for it seems evident that the normal environment of these cells in the embryonic circulation, is in marked contrast to that of most other tissue cells, and for which, as emphasized by Harrison, a coagulated medium appears the more favorable for growth *in vitro*. Appreciating the great sensitiveness of the blood cells to surrounding physical, chemical and thermal conditions, especial care was taken in the cleaning and sterilization of all glass ware and apparatus as well as in the maintenance as nearly as possible of a constant normal temperature throughout the experiments. Variations in culture conditions were studied, including a comparison of free hanging drop cultures with others resting upon a glass surface or a film of agar-agar. Cotton fibers were introduced into some cultures, and Ringer's solution into others. Comparisons were also made between dry culture chambers and moist chambers in the bottom of which various quantities of water, Ringer solution and plasma had been placed. Of these various experiments the cultures in chambers kept moist by a small drop of distilled water proved the most satisfactory. The investigation included over eighty experiments. In each case controls were made consisting of preparations fixed in formalin vapor and stained with Giemsa's mixture. The cultures were always examined in a warm chamber and camera lucida drawings made of the corpuscles under observation.

Further details concerning the preparation of the cultures, plasma, temperature, normal and degenerative cytological characteristics will be more conveniently discussed in the course of the ensuing description.

b. General characteristics of the erythrocytes as first observed in the cultures

In the examination of the preparations when first made, the various types of red blood cells found present consist of the large nucleated erythroblasts with more or less eccentrically located nuclei, non-nucleated plastids varying in size, smaller erythroblasts with a narrow rim of hemoglobin containing cytoplasm, and nuclei apparently free from cytoplasm; the relative proportion of nucleated and non-nucleated elements depends upon the stage of differentiation of the blood used for the cultures, which it may be noted, is by no means always the same for embryos of equal size but taken from different uteri. The cytoplasm of both nucleated and non-nucleated elements is apparently homogeneous in structure and presents a more or less yellowish color, depending upon the degree of hemoglobin differentiation. The majority of erythrocytes present an even rounded contour. Some of the cells, however, may be elongated in a pear-shaped manner with the nucleus at the smaller end, and in others the cytoplasm may extend out in blunt, rounded, somewhat bud-like projection. Occasional nucleated as well as non-nucleated erythrocytes may possess slender, pointed cytoplasmic processes, the probable nature of which has already been considered (pp. 138-140). In most instances the cytoplasm of the cells, aside from certain form changes to be described presently, is more or less quiescent with the exception of the slender pointed processes just mentioned, which may manifest quivering and even oscillatory movements. In the case of the smaller younger erythroblasts where the greater part of the cell may undergo slow changes in form, as may be observed directly in the vessels (fig. 36), these cytoplasmic movements are probably not inadequately described as amoeboid in character. Whether the same conclusion is to be drawn for the oscillatory movements of the slender processes of the more mature erythroblasts is less certain, especially as processes with similar activities may occasionally occur in the non-nucleated erythrocytes.³

³ In regard to this question of amoeboid motion, it may be questioned whether our present knowledge is as conclusive as could be desired as to just when the

c. Derivation of plastids from erythroblasts by a process of cytoplasmic constriction

Circumstances necessitated beginning the culture in the afternoon. Having been made, they were then placed in the incubator and observed as frequently as possible, the more favorable preparations being kept under observation for several hours or even the entire day. During the earlier part of the investigation, attention was especially directed to those erythroblasts which seemed to be most advanced in differentiation as indicated by the increased amount of hemoglobin and the smaller, more compact, pyknotic and eccentrically located nuclei. These cells were carefully observed for continuous periods of time, to ascertain, if possible, steps in the process of their transformation into non-nucleated erythrocytes, and whether the evidence would indicate this process to be in the nature of an extrusion of the nucleus from the erythroblast, or its disintegration and dissolution within the cell body. Biased by the prevalent view that the nucleus eventually disappears from the erythroblast either by an intracellular disintegration or by extrusion, it was with considerable surprise that no conclusive evidence was obtained for either of these alter-

potentiality for amoeboid movement, admitted to be present in the undifferentiated progenitor of the red blood cell, disappears in the course of subsequent cytomorphosis, or whether in certain cases the phenomena may manifest itself in a degree even in the more mature erythrocyte as described by Morris and Thayer ('11), who observed activities in large erythroblasts or megaloblasts in cases of anemia which, after due consideration of hemoglobin content, crenation, osmosis, they interpreted as amoeboid in nature. They also describe the observation of "exactly the same changes in shape in macrocytes (figs. 4-6, 35-37, 39-45, 98-102, 108-112, 113-118, 119-125). The rapidity of change may be quite as great as in the nucleated red corpuscles and may present all the varieties observed in the latter. There was no discoverable difference in kind or in degree. As in the case of the megaloblasts, not all of the macrocytes exhibit amoeboid movements (fig. 11). That non-nucleated cells should do so at all seems strange. But if the motility of the megaloblasts is in reality amoeboid—and to the writers there appears to be no other explanation—then there can be no doubt that certain macrocytes, too, are possessed of it" (p. 6). Cytoplasmic movements apparently amoeboid in character have also been noted by Schultze ('64, p. 358) in the red blood cells of young chick embryos, by Dekhuyzen ('92) in the nucleated erythrocytes of amphibian larvae, and by Ruzicka ('03, p. 306) in the red blood cells of the frog.

natives. On the contrary the non-nucleated elements were found to arise by a process apparently fundamentally different.

The best results were obtained with autoplasmic cultures made from blood taken directly from the heart. Selecting a successful culture in which the erythrocytes present a normal appearance, a careful examination of the erythroblasts, especially of those with the more eccentric, pyknotic nuclei, an occasional blood cell will be found exhibiting a cytoplasmic activity distinguishing it from the surrounding cells. This activity in its earlier stages manifests itself in a modification of the rounded contour of the cell (as seen in a surface view) of such a character as to result in a somewhat pear-shaped elongation of the cell (figs. 13-17, *a*), with the nucleus at one pole of the long axis, and the greater bulk of the cytoplasm tending to segregate itself at the other. The cytoplasm at the latter pole of the erythroblast is observed to be in a state of more or less quivering activity, accompanied at varying intervals by slight elongation and subsequent retraction of the cell body. In other respects such an erythroblast may appear perfectly normal its nuclear, cytoplasmic, and hemoglobin elements apparently differing in no material way from that of the adjacent quiescent, normal nucleated and non-nucleated erythrocytes. The further behavior, which in a favorable case may now be observed in the erythroblast under consideration, is illustrated by the successive stages shown in figure 14. This preparation was a 20-hour blood culture from a 30 mm. pig embryo. The erythroblast to be described was characterized by the slightly elongated form and cytoplasmic activity of the type just described. This active cell was brought into the field of the microscope at 11.40 a.m. and observed continuously until 12.15 p.m. A constriction in the cytoplasm of the erythroblast soon became evident, which within four minutes had assumed the characteristics shown in figure 14, *b*. Gradually this cytoplasmic constriction became more marked (*c*), and within twenty minutes after the observation had been begun the process of constriction was completed (*d*), at which stage the parent erythroblast had now become divided into two parts, the one still containing the original nucleus, the other a non-nucleated hemoglobin-

containing element or plastid. During the remainder of the period of observation these two parts became more widely separated but neither manifested any further changes (stages *e-i*), both now apparently assuming a quiescent condition at least as far as indicated by any visual evidence of cytoplasmic activity. In the present case it will be observed that the two bodies are approximately equal in diameter. In the nucleated portion, the nucleus, which has maintained a constant appearance in structure, size and form throughout the process, is surrounded by a considerable amount of cytoplasm; the cell body is slightly irregular in form with the cytoplasm accumulated somewhat more upon one side of the nucleus than the other. The non-nucleated portion, or plastid, as it may now be conveniently designated, presented a smooth, rounded contour, appeared to be homogeneous in structure, and in both size and amount of hemoglobin could not be distinguished from the average plastids, or non-nucleated erythrocytes, surrounding it in the microscopic field (cf. fig. 13, stage *t*).

Having described a typical case of the origin of plastids by this method, variations in the process as observed in the autoplasmic cultures may now be considered.

First, as to the time involved in the constriction process. In the instance just described it will be noted that an interval of about twenty minutes elapsed between the time of the first observation and the completion of the cytoplasmic constriction. This time varies, however, from a few minutes, or even seconds, to thirty minutes or longer, (figs. 13 to 20), depending obviously, in part at least, upon the stage of the process at which the observations were begun. After some experience the observer tends to become more proficient in selecting erythroblasts at stages of activity which are usually soon followed by a completion of the constriction within a few moments, although not infrequently the characteristic changes preliminary to a constriction may be observed for several hours without the occurrence of any marked progress, the cell sometimes even becoming apparently quiescent again.

Second, as to the time of occurrence in the cultures. Typical cytoplasmic constrictions of the type just described may be found in good cultures at any time during the first two or three days.

They were observed more frequently on the second day than during the first half-day. Their behavior may possibly be comparable in this respect with the similar behavior described by Burrows, Carrel and others in the culture of various tissues in which the processes of growth in the tissue cells were observed to be less evident during the earlier than the later stages of the culture. That the present cytoplasmic constriction of the erythroblast may, however, take place as soon as the cultures are made, is illustrated in the blood of a 30 mm. pig, Experiment 43, *b*. This culture was examined immediately after the completion of the preparation of several cultures included in the experiment, the technique for which had occupied approximately one hour's time. As soon as examined, several erythroblasts in the culture were observed to be at stages of activity indicative of subsequent cytoplasmic constriction. One of these cells was selected for continuous observation, the results of which are recorded in figure 13.

Third, as to the size and other characteristics of the nucleated remainder of the parent erythroblast. In the present observations the amount of cytoplasm remaining associated with the nucleus corresponded more frequently to that shown in figures 13 to 17. The quantity of cytoplasm may, however, vary considerably, so that whereas the amount present may, on the one hand, be occasionally even more than that shown in these figures, on the other hand, it may be reduced to a mere rim of cytoplasm or appear practically, if not entirely absent, especially where the cytoplasmic constriction occurred close to the nucleus (figs. 18 and 20). Usually the nucleus is small and compact in structure. In some cases the lighter appearance of the cytoplasm as seen in the cultures, suggested that this nucleated remainder is possibly not as rich in hemoglobin as the non-nucleated plastid, although this may possibly be in part due to a thinner and consequently more transparent disc of cytoplasm about the nucleus. After the constriction, the nucleated remainder of the cell may assume an apparently quiet condition and manifest no further evidence of change during a period of several hours observation. In other cases the cell body may slowly extend more or less blunt, rounded cytoplasmic processes which may then again be retracted. In some instances

these processes may also be observed to constrict off and become detached from the cell body (fig. 17, *e* to *s*), thus leading to a further reduction of the cytoplasm which in some cases may even result in leaving a practically cytoplasmic-free nucleus.

Fourth, as to the size of the plastids. The diameter of the plastids arising from such constrictions as illustrated in figures 14, 15 and 18 is practically the same as the average non-nucleated erythrocytes of the embryonic pig blood. Other plastids, although similar in origin, may vary greatly in size on either side of this average. Some are much larger (fig. 16), and equivalent in size to the so-called macrocytes of the circulation, others again are much smaller than the average non-nucleated erythrocyte, and correspond in size to the microcytes. Some of the smaller of these plastids which may be about one third or one fourth the size of the average non-nucleated erythrocyte, or about the dimensions of blood platelets, may arise as small cytoplasmic buds or constrictions from a typical erythroblast, or they may be formed as a further subdivision of a larger plastid (fig. 17, *l-s*). In the latter case, the cytoplasm of the large plastid, when first liberated from the parent erythroblast, not infrequently exhibits a quivering modification of its contour, especially at the point where the separation has occurred. A secondary constriction may arise at this region of separation and cut off one of the small elements under consideration, the activity in the remainder of the large plastid then ceases and the corpuscle assumes a stable, quiescent condition. These smaller bodies frequently manifest a considerable degree of activity, as indicated by slight variations in shape, before coming to rest in a more or less stable rounded form.

Fifth, as to the form of the plastids. Since the present experiments consisted chiefly of hanging drop cultures, concerning the determination of the shape of the plastids, an element of uncertainty is introduced on account of the tendency of flattened corpuscles to assume a horizontal position, in which case they may present a deceptively rounded appearance (cf. p. 132). As a rule the plastids whose origin had been directly observed in the cultures, when first formed appeared to have a more or less spherical shape. In order to make more positive observations a further study was made of experiments in which the culture was enclosed

between two cover glasses in such a way as to render it impossible by artificial manipulation, to observe various sides of a given corpuscle.⁴ In such cultures success was attained in observing several cytoplasmic constrictions. The newly formed plastids which under these conditions could be turned over at will, presented a more or less rounded shape in all views.

The question at once arises whether these plastids can be observed in the cultures to undergo any further form changes, and whether there is any evidence of a tendency toward the assumption of the definite biconcave-disc or cup shape. The data bearing on this question are derived from plastids kept under continuous observation for various periods of time, some of them for two or more hours. Usually immediately after its formation the plastid appears somewhat unstable in form as indicated by varying degrees of departure from a rounded contour and readjustments to the original shape (figs. 17, 18). In some cases the plastid would at once, or within a few minutes, assume a fixed and apparently stable form, no further changes being discovered during the period of observation (cf. figs. 14 to 16). In other instances, however, the plastids tend to become flattened and centrally constricted. Sometimes this constriction was observed to result in the subdivision of the corpuscle into two parts, especially in what appeared to be the more unfavorable cultures. That these changes may even in cultures culminate in the assumption by the corpuscle of a cup or bell shape is illustrated in figure 19 from a culture of the blood of a 28 mm. pig embryo in the centrifuged plasma of a 52 mm. embryo. The newly formed plastid in this instance became constricted off from the parent erythroblast at 10.40 a.m., and appeared more or less spherical in shape. Evidence of instability in form, however, soon became evident, and by 11.06 a.m. the corpuscle had

⁴ After considerable experimentation the following method proved the most satisfactory: the culture drop suspended on the under side of a rectangular cover glass was carefully brought into contact with a second smaller cover glass. The latter consisted of a thin circular disc about 12 mm. in diameter, one edge of which had been slightly thickened or bent by heating. This light disc, when brought into contact with the culture medium, was held in place by capillary attraction while at the same time its thickened rim prevented compression of the enclosed blood cells. These preparations were then placed over hollow ground slides and sealed in the usual manner with vaseline.

assumed the appearance shown in the camera drawing (fig. 19, *h*). By 11.47 the plastid had attained a cup shape (fig. 19, *l*). The observations which were continued until one o'clock were made under especially fortunate circumstances through the fact that the corpuscle in question was slowly undergoing a rotation in position. During the latter part of the observations the plastid thus came to occupy a position with the opening of the cup turned directly toward the observer (fig. 19, *m*). It will also be noticed that in the drawing at 11.06 the appearance of the corpuscle is suggestive of changes preparatory to a division into two parts, but inasmuch as the plastid was slowly turning over in the culture apparently what was seen at this stage was a profile view of a biconcave disc, which in the further progress of the turning next presented the circular outline shown in figure 19, *i*. Consequently in this case it appears that the newly formed plastid assumed first a flattened or disc shape before becoming cup or bell-shaped. It is of interest to note that the form changes just described occurred in the homoplastic culture, a fact which suggests that the character of the plasma may be a factor in determining the definitive form of the non-nucleated corpuscle (cf. p. 146). Occasionally, in the final stages of the constriction process, the cytoplasm of the dividing erythroblast may be drawn out in such a manner that with the completion of the constriction, the plastid, instead of having the usually smooth contour of the newly formed corpuscle, may be modified in form by the presence of a slender cytoplasmic process persisting for a time in the region of final separation from the parent cell (figs. 20, 17 and 13.)

3. CRITICAL DISCUSSION OF THE CULTURES AND CYTOPLASMIC CONSTRICTIONS

In determining to what extent we are to regard as normal or abnormal the behavior of the erythrocytes as observed in the cultures, it becomes necessary to consider critically the conditions under which these phenomena occurred, especially in view of the well known fact that blood cells are normally and functionally very sensitive in their reaction to environment.

a. Plasma, temperature and other conditions

The necessity of maintaining the blood cells studied in as normal a medium as possible need not be emphasized. It has already been indicated that the plasma used was from the same embryo whose blood cells were being investigated unless otherwise stated. Since the quantity of blood in 25 to 35 mm. embryos is too small to be favorable for centrifuging, the blood fluid was taken directly from the heart. Through the fact that the percentage of blood cells in a given volume of the plasma is much less in these embryos than in older specimens or adults, together with the tendency of the cells to settle to a lower level, plasma can be obtained which contains a minimum amount of corpuscles. Precautions were taken to obtain this plasma with as little modification as possible. Before removing the embryo from the uterus, the umbilical cord was clamped with a serrefine forceps so as to guard against the possible entrance of foreign fluids into the cut umbilical veins through the suction produced by the beating heart. The cord was then cut and the embryo removed to a sterilized towel, a pad of cotton, or a sheet of filter paper. The amniotic fluid was washed from the embryo by several rinsings with sterilized Ringer's solution (39°), and this solution in turn removed with sterilized filter paper. The heart was then exposed by cutting away part of the thoracic wall and pericardium. Filter paper was again employed to remove the small amount of pleural and pericardial fluids. The heart while still beating was then opened and a small quantity of blood quickly transferred to carefully cleaned, sterile cover-glasses. For this transference several methods were tried including the use of glass rods, glass pipettes and platinum wire loops. The best results were obtained, however, with a small wedge of cardiac tissue, a millimeter or two in length, cut out from the heart wall itself. This piece of tissue was seized with a very fine forceps, immersed in the heart blood and the adhering quantity of plasma and corpuscles brought to the cover-glass. Since the blood corpuscles which are thus transferred with the plasma are sufficiently reduced in number to be favorable for culture purposes, the cover-glass can be at

once inverted, placed over the chamber of a hanging drop slide and immediately sealed with vaseline. The entire operation, occupying but a few minutes, was always performed inside an operating chamber maintained at a temperature of from 38 to 40°. In some cases, as an additional precaution, the air in this chamber was always kept moist by means of evaporation from open vessels filled with warm water. To counteract still further the possible loss of moisture from the cultures, for a part of the experiments, small drops of various fluids (cf. p. 152) were placed in the bottom of the culture chambers. The important fact of the absence of coagulation in these cultures has been previously noted (p. 152).

As already stated the temperature throughout the experiments was maintained at 38 to 40°, both during the preparation and the incubation of the cultures as well as during the microscopical examination of the blood cells. The cytoplasmic constrictions, consequently, cannot be due to excessive heat such as was early described by Schultze ('65) who found that a temperature of 52° caused the non-nucleated erythrocytes to extrude globular processes and beaded filaments which became separated from the original corpuscles, whereas the erythrocytes at a temperature of 38 to 40° remained unchanged.

In the course of a critical analysis of factors which might have artificially produced the constriction phenomena, it suggested itself that since the erythroblasts tend to settle toward the lower surface of the hanging drop, possibly their contact with the surface film of the plasma might result in changes in surface tension sufficient to cause the cytoplasmic constrictions. To test this matter cultures were made in which the contact of the corpuscles with this surface film should be eliminated. After attempting several methods, the most satisfactory results were finally attained with the preparations in which the under surface of the culture drop had been brought into contact with a small circular cover-glass, the rim of which had been slightly thickened so as to prevent any compression of the cells (cf. footnote 4, p. 159). The erythroblasts which were thus resting on a glass surface instead of the culture drop film were observed, however, to undergo cytoplasmic constrictions similar to those occurring in the hanging drop cultures.

b. The erythrocytes: normal and degenerative changes

The question arises as to what extent the blood cells taken from these pig embryos were maintained in normal living condition in the cultures. In the study in vitro of other tissues such as young connective tissue and nerve cells, the vital condition of the cells may in part be determined by the presence of activities such as growth, extension of cytoplasmic processes, amoeboid movement and cell multiplication. In the case of the erythroblasts of the 30 mm. pig embryo, however, it is questionable to what extent manifestations of such vital phenomena as just indicated can be expected in these cells. For it must be taken into account that the mature nucleated mammalian erythrocyte, as pointed out by Minot, represents a late stage in the progressive phase of erythrocytic cytomorphosis, the regressive phase of which involves the formation of the non-nucleated plastid, and that consequently as emphasized by Weidenreich ('03) "Wir dürfen nicht vergessen, dass eben die roten Blutkörperchen der Säuger Morphologisch betrachtet degenerierende Zellen sind" (p. 93). Mitosis and growth in size cannot, therefore, be taken as criteria of life in the mature erythroblasts, for they have already attained their maximum size, nor do they so far as it is at present known, any longer multiply by mitotic division (Minot '12, p. 507). The occurrence of small cytoplasmic processes in the erythroblasts of the 30 mm. embryo has already been described and the evidence considered for regarding them as normally present in the circulating blood of the embryo. These processes may be found for several days in the cultures, although they gradually become less evident.

An observation may also be introduced here concerning young erythroblasts in cultures made from the embryonic liver. It is generally recognized that the young erythroblasts in the early stages of their hemoglobin differentiation may still manifest true amoeboid behavior not unlike that observed in leucocytes. In cultures made from the embryonic liver of the pig, these very young erythroblasts could be clearly seen undergoing slow amoeboid changes in form. This activity could be observed in the

cultures for at least two days, after which it gradually became slower and less evident. As further evidence that the cultures were not unfavorable to the persistence of vital functions may be mentioned the growth of mesenchymal tissue cells occasionally accidentally included in the preparations. These embryonic tissue cells were observed to send out cytoplasmic processes, and give rise to a syncytial network in a manner comparable to the descriptions by Burrows and Carrel for cultures of connective tissue and mesenchymal cells from the chick embryo.

Fixed and stained preparations of the cells in the cultures were made at various intervals during the experiments and compared with control preparations made at the beginning of each experiment. The majority of both the nucleated and non-nucleated erythrocytes in the cultures, including those also undergoing cytoplasmic constriction, do not during the first two days appear to differ essentially in either their cytoplasmic or nuclear stain from that of the controls. The nuclei in some cases appear more compact and take a darker stain; no special tendency toward fragmentation in the nuclei was observed; the hemoglobin of both the plastids and erythroblasts takes a homogeneous hemoglobin stain.

So far attention has been directed chiefly to the evidence indicating the extent to which the normal characteristics of the circulatory erythrocytes may be maintained in vitro. It remains to consider the occurrence of regressive and degenerative changes. The non-nucleated erythrocytes as taken from the circulation must naturally be in various stages of normal physiological degeneration and disintegration. On the other hand, while the life of these corpuscles is presumably of short duration, it is not definitely known just when and how they normally disintegrate (Howell '11, p. 430), and consequently a difficulty is encountered in distinguishing degenerative phenomena in the culture as being of a normal or of an abnormal regressive character. It appears that disintegration of even the nucleated erythrocytes may occur in the embryo. Minot ('12) describes three types of such disintegration: "1, dissolving of the hemoglobin and bursting of the corpuscle; 2, fragmentation; 3, vacuolization, with subsequent plasmolysis" (p. 509). Considering these types with reference to the cultures

of the pig embryo blood, it may be stated that while some hemolysis may be taking place at all stages of the experiments, in the more successful cultures there is little evidence of it until at least after the second day or later, as indicated by the presence of the so-called 'shadow' as well as by the color of the plasma in both fresh and fixed, stained preparations. If proper care be taken cultures can be made containing only a few, if any, crenated cells. Occasionally broken or bursted hemolyzed corpuscles may be found in the older cultures. Concerning fragmentation, those instances in which the corpuscles, whether nucleated or non-nucleated, were observed to become subdivided into a number of smaller pieces approximating the size of blood platelets may possibly come under this classification. Such a fragmentation may also occur in the cytoplasm of the nucleated portion remaining after the separation of the original erythroblasts into two parts. A third degenerative change consisted in the appearance of what were apparently vacuoles in the cytoplasm. In a number of instances this was seen to occur while the given corpuscle was under observation. The movement of one such vacuole has already been described (p. 147). Figure 34 illustrates how such a vacuole may become divided into two parts. In this case it will also be observed that the larger of the two subdivisions apparently broke through the surface of the cytoplasm, although it is possible there still remained between the vacuole and the exterior of the cell a membrane invisible in the unstained corpuscle. It is of interest to note that this behavior of the vacuoles is suggestive of the vacuolization of erythrocytes in the mesenchyme of the human embryo as described by Minot ('12 p. 512, and corpuscle *h*, in fig. 361). All of these degenerative changes are most marked in the older cultures, in homoplastic cultures, and in those experiments in which the plasma had been modified by the addition of Ringer's solution or water.

In résumé it may be said that the behavior and cytological characteristics of the erythrocytes seem to indicate that the culture conditions approximate normal conditions sufficiently to justify the expectation that in such cultures it should be possible to obtain some manifestation of the normal transitional stages in

the origin of the plastids from the mature erythroblasts, especially in blood taken at a time when such changes are at their height in the circulation as in the case of the embryos studied. Concerning the maintenance of the blood cells *in vitro* it is of interest to note that Jolly ('11) who succeeded in keeping leucocytes from the frog's blood alive for nearly twelve months, found these cells still vitally active under conditions in which there had occurred considerable hemolysis and degeneration of many of the other blood corpuscles in the plasma. In his account of the experiment he states:

Dans les tubes, il existait une hémolyse plus ou moins considérable et de nombreuses cellules étaient détruites. Bien que les leucocytes aient trouvé dans ces éléments, qu'ils phagocytent activement, une réserve de nourriture abondante, il est vraiment remarquable de les voir continuer de vivre dans un milieu contenant des produits d'autolyse et qu'au premier abord on pourrait penser leur être nuisible (p. 147-148).

4. EVIDENCE CONCERNING THE ORIGIN OF PLASTIDS IN THE EMBRYO BY A SIMILAR PROCESS OF CYTOPLASMIC CONSTRICTION

a. In the living vessels

Regarding the preceding discussion it is not to be overlooked that the final decision concerning the normal character of the mode of plastid formation observed *in vitro* must be based to an important degree upon the evidence indicating the actual occurrence of the same process within the vascular system of the embryo. Attention has already been called to the observation of the formation of plastids by cytoplasmic constrictions in the fresh cultures immediately after their preparation (fig. 13). Having seen these changes in the blood just removed from the vessels, an attempt was next made to ascertain whether similar processes could be observed within the fresh vessels. For this purpose pig uteri were brought into a warm operating chamber at the slaughter house within a few minutes after the killing of the parent animal. All the operations were carried on within this warm chamber at a temperature approximating 38 to 40°. The procedure consisted in opening the uteri, laying the foetal membrane over a clean slide placed upon the warm stage of the microscope, and the prepara-

tions examined at once. The amnion and its vessels (the later probably chorionic in origin) proved most suitable on account of its transparency. By opening the amniotic sac (while the heart of the embryo was of course still beating), gently arranging the membrane upon a slide and covering lightly with a cover-glass, fairly good views could be obtained of the blood corpuscles gliding along through the vessels and capillaries. Their constant motion rendered it difficult to obtain accurate camera drawings but the nature of the observations is indicated in figures 32 and 33 from a 22 mm. embryo. Figure 32 shows an erythroblast lying within a medium sized vessel, the walls of which are indicated in outline. It will be observed that the erythroblast drawn has become almost completely sub-divided into two parts, the one nucleated, the other non-nucleated. It was not possible to follow this cell long enough to ascertain the completion of the process. In the case illustrated in figure 33, in which the erythroblast came under observation just before a constriction occurred, circumstances fortunately favored the continuation of the observation for several minutes. When first seen at 4.02 p. m. the cytoplasmic pole of this cell manifested activities tending toward a subdivision of its cytoplasm, to all appearances comparable in every respect with the constriction behavior already described in the cultures. Four minutes later the constriction was completed and hemoglobin containing plastid had become entirely separated from the parent erythroblast.

b. In fixed vessels

Figures 5 and 6 are camera drawings of erythroblasts found in vessels which had been carefully fixed with Zenker-formalin at a temperature of about 38°. The vessels selected for study were those occurring in the membranes surrounding the amniotic fluid. These membranes were fixed in situ before removal from the uterus, then stained, cleared, and suitable pieces mounted in toto. If one examines carefully the nucleated erythrocytes within these vessels cells are occasionally found having the characteristics indicated in the figures, in which there is a decided constriction of the cell body in the region between the nuclear and cytoplasmic poles.

In other respects these cells appear normal. The cytoplasm takes a homogeneous hemoglobin stain. The nuclei are relatively small and compact, and present a characteristic dark stain. In a word, these erythroblasts present the appearance of having been fixed at a stage in the process of constricting off a portion of the hemoglobin-containing cytoplasm. The objection might be raised that these forms instead of really representing stages in the formation of plastids are merely the result of cells having been fixed while compressed within the walls of narrow capillary vessels, since as is well known erythrocytes are quite labile and readily adapt themselves to passage through narrow spaces. While some irregularities in the form of the fixed corpuscles may be due to such factors, a number of considerations do not appear to permit the disposal of the constriction forms just described in this way. In the first place if these forms are due to compression within capillaries it seems justifiable to expect their occurrence in considerable numbers, especially in these membranes which contain numerous capillary networks. On the contrary, however, their frequency of occurrence is comparable to that of the telophase of mitosis in the erythroblasts, so that it is necessary to search rather carefully to find such constriction cells. Nor does this frequency appear to vary under different conditions of fixation. For example, since in some of these membranes fixation *in situ* took place while the heart was beating, the precaution was taken to cut the cord carefully just previous to fixation and thus eliminate a possible congestion of the blood cells and consequent increased pressure in capillaries as they were being penetrated by the fixing fluids. However, no material difference was noted in either method of fixation. Second, compression in a uniform capillary tube would seem to tend toward the production of a cylindrical elongation of the cell rather than a constriction; while on the other hand, mutual compression of a number of corpuscles packed together in a larger vessel tends towards the assumption of angular or irregular rectangular forms, as may be observed in instances of congestion. Third, the constricted forms may be found in both large and small vessels, in association with erythroblasts undergoing various phases of mitosis, and under conditions where it

does not seem probable that they had been subjected to abnormal pressure. In the case of figure 5, for example, the cell in question was lying in a vessel having a diameter several times that of the blood corpuscles, at a considerable distance from any capillaries, and under conditions in which the surrounding blood cells were not closely crowded. Fourth, the erythroblasts with these cytoplasmic constrictions are further characterized as having the smaller more compact nuclei typical of the mature cell, whereas similar constrictions were not observed in younger undifferentiated erythroblasts with large nuclei.

c. Size and form of the plastids

Some attention has already been given to the variation in size and form of the non-nucleated erythrocytes found normally in the circulation of 25 to 35 mm. pig embryos (cf. p. 149 and figs. 4, 11, 26 to 31). From their size it seems self evident that the larger plastids must have been derived from the large erythroblasts; (as for the question which some investigators have raised to the effect that the first erythroblast do not give rise to non-nucleated elements see discussion on pp. 176-177). At the other extreme are hemoglobin-containing elements about the size of blood platelets or even smaller, which it appears must have arisen as subdivisions of a larger erythrocyte. In form both the large and small plastids vary from cup-shape to biconcave discs in both fixed and fresh preparations, with apparently a preponderance in the direction of the cup or bell form (figs. 4, 29, 30). The fact that even the smaller plastids may also present a disc or cup shape is of interest since it appears highly probable that they must have arisen as subdivisions of a larger corpuscle, whether that process be one of fragmentation or of cytoplasmic constriction of the character under consideration. That small cytoplasmic subdivisions of erythrocytes may take on the typical form of the definitive plastid has been noted by Erelch ('85) who finds that the so-called poikilocytes may assume "eine deutliche Dellung" and concludes "dass in dem abgesehnürten Theil neben dem Hämoglobin das Discoplasma vorhanden ist,

dem die Neigung Scheiben von bestimmter Form zu bilden, auch in seinen kleinsten Partikelchen innewohnt;" (cf. also Erlich and Lazarus '09, p. 67-68, and Weidenreich '03, p. 47-55).

Not all the plastids, however, have this biconcave-disc or cup shape. As a rule the smallest and not infrequently even the largest ones are more or less spherical (figs. 27, 28, 8 *b*), as may be positively ascertained by mechanically rotating the fresh unfixed corpuscles. In this respect they correspond with many of the newly formed plastids observed in the cultures. It is of interest also to note that in the human embryo "the early human plastids do not have the characteristic form of the definitive corpuscle, but retain the spherical shape" (Minot '12, p. 509).

Finally, a more or less spherical plastid is occasionally found having a single fine hair-like process (fig. 8). This process, which seems unquestionably a part of the cell cytoplasm, drawn out in this attenuated form, closely resembles the similar processes which were occasionally observed to arise in the cultures, where in an as yet uncompleted cytoplasmic constriction the intervening cytoplasm had become drawn out into a fine, almost invisible thread (figs. 13, 20; cf. also p. 138). It seems difficult to escape the conclusion that these cytoplasmic processes in the circulating plastids have also arisen in connection with a process similar to that observed *in vitro*.

d. Erythrocytic nuclei after plastid formation

Free erythrocytic nuclei deficient in surrounding cytoplasm have of course long been recognized and their presence in the embryonic circulation advanced as evidence for the enucleation of the erythroblast by a process of nuclear extrusion. In view of the results of the present study, it may be questioned, however, whether the latter conclusion follows necessarily. In the first place, it will be recalled that in certain cases cytoplasmic constrictions were observed in the cultures in which the process took place in such a manner as to leave a practically if not entirely cytoplasm-free nucleus. Second, instances were noted in which the cytoplasm surrounding the nucleus remaining after a com-

pleted constriction, subsequently fragmented off, thus leaving an apparently cytoplasm-free nucleus. The occurrence of the so-called free erythrocytic nuclei does not, therefore, appear to be conclusive as regarding either the question of nuclear extrusion or of cytoplasmic constriction.

The occurrence, however, of similar nuclei plus a small quantity of hemoglobin-containing cytoplasm would be evidence of a more decisive character. Figures 10 *n*, 11 *n*, 12 *n*, represent small nucleated corpuscles in the embryonic pig blood which were if anything more frequent than the free erythrocytic nuclei. The cytoplasm, which varies from a narrow rim around the nucleus to a larger quantity as illustrated in the drawings, takes a hemoglobin stain with Giemsa's fluid. The nuclei are small, compact, and in stain and structure appear indistinguishable from either the nuclei of the fully differentiated erythroblasts or from the free nuclei. Some of these nuclei also present a flattened form (figs. 11, 12) similar to the flattened nuclei previously described for the erythroblasts (p. 135). These structural characteristics do not favor regarding these cells as young undifferentiated erythroblasts. On the contrary, their size, form, nuclear and cytoplasmic structure and stain conform cytologically with the nucleated portions remaining after the completion of cytoplasmic constrictions in the cultures, and what should be expected in the blood of the embryo if a similar process occurs normally within the embryonic circulation.

In studying the literature bearing on the present subject it is important to note that while Howell ('91) failed to find in the cat embryo erythrocytic nuclei with a small quantity of cytoplasm such as just described in the preceding account, on the other hand, Jolly ('07) states his observation that "*dans certain cas, comme chez le rat, le pore, sur beaucoup de globulés, le disco-plasme apparaît comme réduit, si bien qu' on pourrait se demander si on à affaire à des noyaux expulsés avec une certaine quantité de protoplasme hémoglobique*" (p. 207).

As for the fate of these nucleated remainders the data at hand does not justify a conclusion as to whether some of them, at least such as may have an appreciable quantity of cytoplasm, may

persist, grow and again participate in the formation of new plastids, whether they may possibly become cells of a lymphocytic type, or whether, as is in accord with the more generally accepted view, they are in the last stages of a cytomorphosis, which is soon to culminate in their death and disappearance. While the latter alternative seems at present the more probable, the force of one of the usual arguments supporting it based upon the reduced size and compactness of the nucleus appears less convincing, so far as this nuclear condition is concerned, if one considers that in the cytomorphosis of the spermatocyte the small compact nucleus of the spermatid, together with the reduced amount of cytoplasm remaining at the end of the process is by no means indicative of ensuing deterioration and cell death.

In conclusion, then, concerning the erythrocytes of the pig embryo, the occurrence in carefully fixed vessels of erythroblasts having a constriction of the cytoplasm between the nuclear and cytoplasmic poles of the cell, the variation in the size and form of the plastids, the presence of pyknotic erythrocytic nuclei surrounded by a rim of hemoglobin-containing cytoplasm, and the observation in fresh vessels of the separation off of a cytoplasmic part of the erythroblast by a process of cell constriction, is evidence strongly indicating the origin of plastids within the circulatory system of the embryo by a process of cytoplasmic constriction similar to that observed in the cultures.

DISCUSSION CONCERNING THE ORIGIN OF NON-NUCLEATED ERYTHROCYTES

1. THE QUESTION OF NUCLEAR EXTRUSION AND INTRACELLULAR DISINTEGRATION

As is well known there are two prevalent views concerning the enucleation of the mammalian erythroblast. The one, which traces its origin to Kölliker ('46) being that in the transformation of erythroblasts into non-nucleated corpuscles the nucleus is dissolved within the cell, the other, originating with Rindfleisch ('80) that the nucleus is expelled from the cell. In spite of the subsequent accumulation of an extensive literature for both these views, in the words of Lazarus and Naegeli ('09) in Erlich's work

on Anemia, "the investigations of the last ten years have failed to reconcile the champions of these doctrines. . . . Suffice it to mention that the names of E. Albrecht, Howell, M. Heidenhain, Vd. Stricht, and Jünger have appeared in support of Rindfleisch; while Massloff and Naegeli have recently taken up the cudgels for Neumann and Kölliker" (p. 73). Since, however, the greater number of recent investigators, including Jolly, Maximow and Weidenreich incline toward the view of nuclear extrusion, some of the evidence for the latter conclusion may accordingly be here briefly discussed, confining our attentions chiefly to conditions in the embryo.

The following facts are among those which have been advanced as evidence for nuclear extrusion. First may be mentioned the presence of free nuclei in the circulation. Granting that a given free nucleus is erythrocytic in origin, for the conclusion that it must have originated by nuclear extrusion, two other alternatives have already been presented, namely, that the nucleus under consideration could have separated from the erythroblast by a cytoplasmic constriction so close to the nucleus as to leave it practically, if not entirely, free from any surrounding cytoplasm, or that the nucleus plus a small quantity of cytoplasm as originally separated from the parent cell had subsequently lost this cytoplasm by fragmentation.

A second argument for nuclear extrusion has been based upon the occurrence of free erythrocytic nuclei ingested by phagocytic cells. Concerning this point it is readily appreciated that if the objections just raised in connection with the subject of nuclei free in the circulation are valid, they apply with equal force to the interpretation of the ingested nuclei found in the phagocytes. Furthermore it is not to be overlooked that the entire erythroblast as well as free nuclei may be phagocytized, as has been described and figured by Maximow ('09) for the rabbit embryo (pp. 480 and 545). The writer has also observed the same phenomenon in phagocytic cells of the pig embryo. A dissolution or digestion of the cytoplasm before that of the nucleus in such an ingested corpuscle would leave an uncertainty as to whether a given nuclear inclusion represented a previously cytoplasmic free nucleus.

Third, considerable evidence has accumulated demonstrating that the erythrocytic nuclei may, while still within the erythroblasts, undergo in some cases a process of fragmentation or budding off of one or more nuclear particles, as described for example by Howell ('91) in the cat after hemorrhage. Nuclear particles occasionally found in plastids have accordingly been explained by various investigators, including Howell ('91), Weidenreich ('04, '06), and Jolly ('07), as nuclear fragments remaining after the expulsion of a part of the nucleus and their presence has consequently been regarded as an argument for nuclear extrusion. On the other hand, Pappenheim ('95) among others, has utilized these particles as evidence for intra-cellular nuclear disintegration rather than for extrusion. However, here again it may be questioned whether this datum can be considered conclusive for either view, for a third possibility remains concerning these particles, namely, that in case a portion of the nuclear material had budded off from the erythroblast nucleus, subsequent division of the cell into two parts by a process of cytoplasmic constriction may take place in such manner as to include the nuclear particle in the newly formed plastid and thus account for the origin of non-nucleated erythrocytes containing remnants of nuclear material. In the pig embryo, at least according to the present observations, these nuclear particles are of infrequent occurrence and no plastids containing such particles were observed in the cultures. In triton, however, Jolly ('07) describes an authentic case of the formation of a nuclear bud in the nucleated red blood corpuscle and the inclusion of this nuclear fragment in a portion of the cytoplasm subsequently constricted off from the nucleated cell which he describes as follows:

On peut observer des fragments spheriques contenant un corps colorable par les couleurs basiques; ce corpuscule est a fragment nucléaire, un bourgeon nucléaire qui s'est isolé et qui s'est trouvé séparé de la cellule avec le fragment. Il ne s'agit pas là d'altérations artificielles dues aux réactifs; le phenomene peut être observé sans le secours de réactifs (p. 195).

The indirect evidence for nuclear extrusion just considered, then, is of such a character that for a final solution of the problem it appears that recourse must be made to the direct evidence for the actual process of enucleation as drawn from the study of fixed

and fresh material. Turning to the data in the mammalian embryo bearing on this subject it appears that conclusions for nuclear extrusion are not so self-evident as might be anticipated.

The first erythroblasts of the embryonic circulation are, as is well known, much larger than those found in both later embryonic life and the adult. The direct evidence for the mode of enucleation of these large erythroblasts, or megaloblasts as they have been designated by Erlich, appears to be inconclusive and has given rise to a diversity of opinion. Erlich (Erlich and Lazarus '09, p. 74) holds that the nuclei of these megaloblasts are dissolved within the cell in contrast to the so-called normoblasts in which he supposed nuclear extrusion to obtain. Weidenreich ('05) on the contrary questions the validity of this conclusion and maintains that the nuclei are extruded from the erythroblasts of the embryo as well as from those of the adult (p. 434; cf. also Weidenreich '11, p. 45). Jolly ('07) was unable to find any stages in the expulsion of the nuclei of these embryonic cells in either the circulation or the liver of the embryo and concludes that the nuclei probably disappear chiefly by atrophy within the cell (p. 278), although at the same time he holds that in the adult the process of enucleation is one of extrusion. Maximow ('09, p. 478) states that the early red blood cells in the rabbit embryo manifest little tendency toward enucleation although he reports some evidence for extrusion in the case of the embryo guinea pig.

The direct evidence for nuclear extrusion in the embryo thus appears inconclusive, if not contradictory, indeed to such a degree that several investigators have even raised the question whether the erythroblasts in the young embryo ever differentiate into non-nucleated corpuscles. However, it must be emphasized that it does not seem that there can be any reasonable doubt, at least for the pig embryo, of the occurrence of large non-nucleated erythrocytes which could have arisen only from the large nucleated red corpuscles or megaloblasts. It will be observed that the same fact has also been recognized in the preceding references from both Jolly and Weidenreich (p. 65). On the other hand, the problem of just how these plastids are derived from the megaloblasts appears still to be an open one, concerning which the

present evidence for their origin by cytoplasmic constriction may, therefore, be justifiably introduced for consideration.

In the foregoing review of the evidence for nuclear extrusion it becomes evident that conclusions supporting this view are based primarily on data drawn more largely from observations of the vascular system of the adult and on the embryo approaching term. Since the present study deals only with embryos up to 40 mm. in length, a discussion of the extensive data and literature for nuclear extrusion in the bone marrow of older embryos and the adult on the basis of the present observations is not warranted. However, it is of interest to note that the erythroblasts of the bone marrow of the adult, from which the data under consideration have been derived, are chiefly of the smaller type or so-called normoblasts. Consequently, if the conclusion for the origin of plastids in the embryo by cytoplasmic constriction be correct, it remains to be determined whether possibly the apparent nuclear extrusion described for the bone marrow of the adult may not be fundamentally a process of cytoplasmic constriction in which, on account of the smaller size of the erythroblast, stages in this process may present the appearance of an extrusion of the nucleus, whereas in the case of the large erythroblasts of the embryo, with a greater quantity of cytoplasm, the real character of the process is more clearly evident.⁵

Before proceeding further in discussion, a digression is desirable concerning the erythroblasts of the early mammalian embryo. Erlich ('80) and a number of subsequent investigators have sought to make a distinction between the larger nucleated erythrocytes or megaloblasts of the embryo and the smaller nucleated corpuscles or normoblasts of the adult, and have maintained that they are genetically distinct in both their origin and mode of differentiation. In the present study of the pig embryo no substan-

⁵In some of the figures which various investigators have given as evidence for nuclear extrusion, emphasis has been placed upon the compressed or elongated form of the nucleus as indicative of its being forcibly squeezed or pressed out of the cell body. There appears, however, to be some ground for the question whether the possibility has been eliminated that some of these may be instances of merely profile views of nuclei already flattened in the course of normal differentiation previous to the formation of the plastids, as has been shown to occur in the pig embryo (cf. figures 22, 45, and pp. 135, 142.)

tial evidence has so far been obtained indicating the correctness of such a distinction and it has been assumed that these large red blood cells are not fundamentally different from normoblasts in their mode of differentiation, both types giving rise to non-nucleated erythrocytes. It is important to note that the more recent investigators arrive at a similar conclusion for mammals in general. Weidenreich ('05) describes megaloblasts and normoblasts of the embryo as differing merely in the size of the cells, rather than in anything more fundamental in character, and concludes that

Es besteht keine Trennung der Formen im Erlich'schen Sinne (p. 434). Der fötale Erythrozyt variiert in seiner Grösse beträchtlicher als der postfötale; neben ganz grossen Formen (Gigantozysten) finden sich in fötalen Leben grosse (Makrozyten), normale (Normozyten) und auffallend kleine (Mikrozyten), die aber alle durch Übergänge verbunden erscheinen. Das gleiche gilt aber auch für das kernhaltige Stadium; es gibt Gigantoblasten, Megaloblasten, Normoblasten und Mikroblasten und dazwischen gleichfalls alle Übergänge. Streng genommen müsste man also alle diese Formen als besondere Typen auffassen (p. 449).

Jolly ('07) fails to find sufficient ground for Erlich's distinction; "nous pensons que cette opinion résulte surtout d' une confusion, et que, en voulant faire, avec Erlich, deux espèces spéciales des normoblastes et des mégaloblastes, on risquerait de se tromper" (p. 276). Minot ('12) reaches a similar conclusion, as indicated in the following reference to Erlich's classification.

Weidenreich expresses himself positively against this opinion, justly it seems to me. In fact, the mesamoeboids in young embryos vary much in size (compare fig. 354) and a similar unevenness prevails also among the ichthyoid cells (fig. 357). It is further probable that the large mesamoeboids, of which the majority form small cells by continual division, in small part at least develop haemoglobin precociously and thus produce the so-called megaloblasts (p. 507).

2. EVIDENCE FROM PREVIOUS INVESTIGATORS BEARING ON THE ORIGIN OF PLASTIDS BY CYTOPLASMIC CONSTRICTION

a. In mammals

After having made the observations described in the present paper indicating the origin of plastids by cytoplasmic constriction, it was later found in an examination of the literature on erythrocytes that the possibility of such an origin for these red corpuscles

had been advanced and discussed at several times in the history of the subject, but through a lack of sufficient evidence the theory has in more recent years received but little serious consideration. Attention may be called first to the nature of the data which Rindfleisch ('80), to whom the origin of the theory of nuclear extrusion is generally traced, himself advanced in support of the extrusion view. Although it seems Rindfleisch has been credited with saying that the nucleus is extruded from the cell free from any cytoplasm such is not his original statement. On the contrary he describes (p. 33) and figures the nucleus which has left the cell as surrounded by an appreciable quantity of cytoplasm. It is a fact worthy of note that if one examine his drawings in figure 6 for the embryo of the guinea pig illustrating stages in nuclear extrusion, it is surprising how nearly they correspond in appearance to processes of constriction described for the pig embryo. To one who has observed the constriction process in the living erythroblast the similarity in this figure is sufficiently evident to suggest that what Rindfleisch described from fixed preparations as extrusion might possibly have been explained in terms of cytoplasmic constriction.

Indeed, contemporaneously with Rindfleisch, just such an hypothesis was advanced by Malassez. In 1878 in a study of the spleen, and more fully in 1880 and 1881 in the bone marrow of the rabbit, deer, calf, cat, and man Malassez carefully described and figured for erythroblasts bud-like elongations of the cytoplasm, which, although without having observed the actual process, he assumed subsequently to separate from the parent cell and become non-nucleated red corpuscles: "Pour moi donc, ces éléments ne se transforment pas en globules rouges par suite de la destruction ou de la sortie de leur noyau; ils conservent leur individualité, leur protoplasma produit un bourgeon qui, venant à se détacher, forme un nouveau globule" (p. 15). Engel ('93, '98 and '99) from studies of mouse, pig, and human embryos drew the conclusion, reaffirmed in 1906, that there occurs in erythroblasts a mode of direct division separating the original cell into two parts, the one nucleated, the other non-nucleated. Similarly Janosik ('02), although more data and figures could be desired in his evi-

dence, referring to the erythroblasts in the blood of spermophile and pig embryos states that

De leur cytoplasm se détachent de petits morceaux qui fournissent les globules rouges définitifs, ou plastides lesquels en circulant dans le sang s'imprégnent plus fortement d'hémoglobine. On rencontre aussi de plus grands fragments de cytoplasma dépourvus de noyau mais colorés par hémoglobine. Ce sont les débris d'érythroblastes dont le noyau, entouré d'un peu de protoplasma, s'est détaché, formant ainsi quelquefois les soi-disant 'noyaux libres', en réalité de petites cellules ayant très peu de cytoplasme. Ce cytoplasme anucléé conserve sa taille primitive ou bien se brise de nouveau en produisant des globules plus petits (p. 280).

Howell ('91), who has strongly supported the nuclear extrusion view, records an observation on the bone marrow of the cat which is especially interesting in relation to the present subject. He says:

In several instances, when examining the marrow, I have met with appearances which seemed to justify Malassez's theory. Nucleated red corpuscles were seen with one or more non-nucleated corpuscles apparently budding out from them I was at first inclined to believe that we must admit that, under certain circumstances at least, new red corpuscles may be produced by budding in the way described by Malassez (p. 104).

In a summation of his discussion he concludes, however, that "the apparent gemmation of non-nucleated red corpuscles from the nucleated forms, as observed by Malassez, is probably owing to the multiplication of the nucleated cell and the subsequent loss of a nucleus from one or more of the daughter-cells before the complete separation of the cells has been effective" (p. 113). Being convinced of the correctness of nuclear extrusion, he states, "the explanation that I have adopted seems to me to be preferable to supposing that in the marrow new blood corpuscles are formed from the same cells by two entirely different methods of reproduction" (p. 104). In an examination of Howell's figure one cannot fail to be impressed with their resemblance to some of the cell forms observed in the present study of the pig embryo in which it was of course possible to ascertain that the cytoplasmic portion which was being constricted off from the cell had at no time contained a nucleus.

b. In other vertebrates

As is well known, the definitive red blood corpuscles of the lower vertebrates in contrast to that of adult mammals are typically nucleated cells. On the other hand, it appears that non-nucleated erythrocytes are normally present even in the blood of some of these lower forms and furthermore that these elements may play a role in respiratory functions. The following observations concerning the origin of these non-nucleated elements are of especial interest with relation to our present subject.

Giglio-Tos ('97) described for the blood of the Lamprey the division of the red blood cells into nucleated and non-nucleated parts, and designated the process as one of 'merotomie.' He regarded the process as a purely physical one which, however, he concluded had the important function of increasing the surfaces concerned in gaseous exchange. In 1899 Giglio-Tos was also able to confirm Eisen's ('97) earlier observation of the occurrence of a similar process in *Batrachoseps attenuatus*, concerning which he states his opinion that these non-nucleated elements take part in respiratory function as well as the nucleated erythrocyte. For triton, Jolly ('04) describes a process of cytoplasmic constriction in the erythrocytes referring to which in his recent work ('07) on erythrocytes, he says:

J'ai montré (1904) que, chez le Triton, au moment de la régénération du sang après le jeûne, le protoplasme de beaucoup de globules prêts à la division forme un gros bourgeon pédiculisé se séparant du fragment contenant le noyau. Non seulement on trouve tous les stades de cette transformation, mais on voit dans la préparation, quelquefois nombreux, libres au milieu des cellules, ces gros fragments protoplasmiques sphériques et sans noyau (p. 195).

which as he states is not to be regarded as due to artificial conditions. Jolly ('04) referring to Giglio-Tos' observations remarked that:

En présence de ce phénomène, très distinct de la dénucléation, expulsion du noyau, on ne peut s'empêcher de penser immédiatement au bourgeonnement protoplasmique des cellules de Neumann, bourgeonnement découvert par Malassez, en 1882, dans la moelle des jeunes Mammifères, et qui pour lui est l'origine des globules rouges sans noyau,

although he makes the reservation "Mais nous ne voulons pas aujourd' hui insister sur ce rapprochement, car nous ne pouvons encore nous prononcer sur la véritable valeur de ce phénomène, dans l'objet que nous étudions" (p. 485). Engel ('94) records the occurrence of non-nucleated red blood corpuscles in the early chick embryo. Engel ascribed their origin to the pinching off of portions of the cytoplasm of the nucleated erythrocytes, a conclusion which he has reaffirmed in 1906 as follows: "In vielen Fällen, sowohl beim Hühnchen als beim Frosch, hing der kernhaltige Teil durch eine protoplasmatische Verbindung noch mit den kernlosen Teile zusammen. Es bedarf wohl kaum besonderer Erwähnung, dass in diesen Fällen von einem Präparationsfehler oder einem Kunstprodukt keine Rede sein kann" (p. 146). Dantschakoff ('08), while not attributing any especial significance to the phenomenon other than its being probably degenerative in character, also notes the occurrence of non-nucleated red corpuscles in the embryo chick.

Though granting the necessity for further investigation, nevertheless, these results lend increased interest to the observations for mammals, for if these non-nucleated corpuscles occurring occasionally in lower vertebrates are to be regarded as precursors of the respiratory elements which were to predominate in the circulatory system of the adult mammal, their mode of origin in the lower vertebrates may throw light on the nature of the process in the higher forms. If the mammalian embryo is in many respects morphologically and phylogenetically comparable to ancestral vertebrates, the evidence for the occurrence of processes of cytoplasmic constriction in the origin of hemoglobin-containing plastids common to both groups of animals may be a matter of more than passing significance.

3. THE RÔLE OF CYTOPLASMIC CONSTRICTION IN THE ORIGIN OF OTHER NON-NUCLEATED MORPHOLOGICAL ELEMENTS OF THE MAMMALIAN BLOOD

During the last few years evidence has accumulated indicating that the constriction off of non-nucleated cytoplasmic portions of cells may play an important rôle in the formation of several

morphological elements of mammalian blood. A well substantiated conclusion is that of Wright's ('10), that "all of the blood platelets are detached portions or fragments of the cytoplasm of the megakaryocytes" (p. 8). Weidenreich ('12) observed the pinching off of portions of the cytoplasm of lymphocytes in the human thymus, which he describes as follows: "Zweitens kommen Partien in der Rinde vor in denen die Rindenzellen grossere oder kleinere Teile ihres Protoplasmaleibes abschneiden, die dann als basophile blutplättchenähnliche Gebilde frei zwischen den Zellen liegend angetroffen werden" (p. 2602). Pappenheimer ('13) in his study of cultures of thymic tissues, found in the thymic cells a

separation of portions of proplasm, which gradually become constricted off and are set free in the plasma. The separated portions contain fat drops and granules. The process resembles curiously the formation of blood platelets from megakaryocytes, as first described by H. Wright. Its significance here is uncertain; it may be found in cells which show no other degenerative changes (p. 313).

Weidenreich and Goldmann ('12, p. 61) found similar changes occurring in plasma cells.⁶ Downey and Weidenreich ('12) describe the same process for lymphocytes in normal lymph glands. In a recent investigation Downey ('13) further "found that the lymphocytes of the germ centers and pulp of the spleen were fully as active in the cutting off particles of their cytoplasm as were those of the lymph glands" (p. 40), and, moreover, after careful study draws the important conclusion, "that the hyalin bodies which are constricted off from the protoplasm of lymphocytes and mononuclears, especially in the rabbit, are in no way related to blood platelets, as was claimed by Dominici and others" (p. 52).

We have then what appears to be convincing evidence that megakaryocytes, plasma cells and lymphocytes, and thymic cells (i.e., if the latter are to be regarded as distinct from lymphocytes) contribute at least two important non-nucleated morphological elements to the mammalian blood, and that they do this by con-

⁶ Compare also a recent paper by Brown ('13) for the constriction off of parts of the cytoplasm from "hyperplastic endothelial cells in the marrow, and mononuclear and transitional cells (premegakaryocytes) in the marrow, spleen, and blood." (p. 286).

stricting off parts of their cytoplasm and liberating them into the vascular system.

The occurrence of this process in these three types of cells is more than ordinarily interesting on account of their apparently close relationship genetically. Weidenreich ('11) has already called attention to this comparison in his discussion of cytoplasmic constriction in these cells in his statement that:

Die Tatsache, dass von jenen Zellen Plasmabestandteile wieder an die Umgebung abgegeben werden, spricht dafür, dass hier eine wirkliche Umarbeitung stattgefunden hat. Überhaupt ist die Abgabe von Substanzen durch lymphocytäre Zellen eine weit verbreitete Erscheinung; sie lässt sich nicht nur bei gewöhnlichen Lymphocyten im Bindegewebe beobachten, sondern sie geht, wie Dominici gezeigt hat, auch in grossen Umfange bei bestimmten Tieren in Lymphdrüsen und Milz von sich und sehr wahrscheinlich haben wir auch—die neueren Untersuchungen Wright's sprechen zugunsten dieser Annahme—in den Blutplättchen der Säuger Abschnürungen der Knochenmark—Riesenzellen zu sehen, die in die Zirkulation gelangen und dort der Auflösung anheimfallen. Da die Riesenzellen selbst aus den lymphocytären Formen des Knochenmarks hervorgehen, wären diese Abschnürungen in Parallele zu setzen zu jenen der Lymphocyten und Plasmazellen (p. 60).

In view of the data just discussed concerning the origin of plastids, may not similar considerations be extended to the cytomorphosis of the mammalian erythroblast? Recent morphological investigators of the blood support the monophyletic view involving the conclusion that erythroblasts, and this group of plasma cells, megakaryocytes and lymphocytes are genetically closely related, having a common origin either directly or indirectly from mesenchymal cells. Consequently it would not appear remarkable if it should be found that in both groups of cells similar processes are involved in the contribution of morphological elements to the blood.⁷ That whereas in lower vertebrates the hemo-

⁷ In connection with the subject of the formation of hemoglobin in the cytoplasm of the mesenchymal cell and its ultimate detachment from the nucleated remainder it is not without interest to note that Schäfer ('74) and also Ranvier ('74) described the development of hemoglobin within the cytoplasm of young connective cells from which it was later liberated into the circulation in the form of non-nucleated corpuscles. Maximow ('09, p. 513) also records the observation in endothelial cells of what appeared to be the constriction off of hemoglobin containing portions of their cytoplasm. More recent investigators, while confirming the observations, have not been able to accept the conclusion drawn from them by

globin tends to remain enclosed in the cells in which it is first formed, in mammals on the contrary, the mesenchymal cell differentiating into the erythroblast, accumulates hemoglobin in its cytoplasm, the nucleus assumes a more or less eccentric position, physiological and structural changes ensue which result in the initiation of a process which constricts off the mass of hemoglobin containing cytoplasm, liberating it into the circulatory system where it functions as a free non-nucleated red blood corpuscle. The fact that the process is one of cytoplasmic constriction is apparently more clearly evident in the case of the larger erythroblasts of the embryo where the nucleated remainder may include a perceptible quantity of cytoplasm, as in the pig embryo, than in the smaller erythroblasts of the adult organism where the process may not be readily distinguishable from one of nuclear extrusion, even though the two phenomena may be fundamentally different.

SUMMARY AND CONCLUSIONS

The observations and conclusions drawn from this study of the erythrocytes of the pig embryo may be summarized as follows:

1. Certain cytological characteristics of the erythroblasts

1. Morphological changes during cytomorphosis.

During their differentiation the erythroblasts pass through a series of successive transitional changes in which:

a. The originally more or less spherical cell body changes to a flattened biconcave disc or even a concavo-convex cup form, so that in the pig embryo, in contrast to the usual descriptions for mammals, the erythrocytes may assume the definitive form even while still nucleated.

Professor Schäfer, and have interpreted the intra-cellular phenomena as instances of atrophy rather than progressive differentiation. Schäfer in 1913 reaffirms his views and observes that "there is no improbability in supposing that in these situations other cells than those which are bodily transformed, with extrusions or atrophy of their nuclei, into erythrocytes, may share in the process of haemoglobin formation, and the haemoglobin infiltrated cytoplasm may become budded off from the cell from which it had been produced. Such budding off of colored cytoplasm from an endothelium cell of a blood vessel is depicted by Maximow in figure 569, *c*, and figure 571, *e*, p. 380.

b. The nuclei, in correlation with these changes in cell form, decrease in size and become structurally compact, assume a more or less eccentric position at one side of the cell, designated in this description as the nuclear pole in contrast to the opposite or cytoplasmic pole, and manifest a tendency toward a flattening in conformity with the shape of the cell body.

2. Cytoplasmic processes:

a. Not infrequently an erythroblast is encountered characterized by a small, pointed, cytoplasmic process projecting from the surface of the cell.

b. Observations were described indicating: first, that these processes are normally present in the embryo, and second, that they may be primarily due to certain conditions attending the final separation of the two daughter cells during mitotic division.

3. Orientation with reference to gravity:

It was discovered that freely suspended mature erythroblasts having eccentrically located nuclei tend to adjust themselves in such a manner with reference to gravity that the nuclear pole of the cell swings undermost.

4. Reaction to osmotic changes:

Under certain changed osmotic conditions such as an increase in the percentage of water in the plasma, instead of the ensuing swelling reaction in the disc shaped erythroblast being equal throughout the cell body, the cytoplasmic pole of the disc becomes swollen and approaches a spherical shape, while the nuclear pole of the corpuscle may undergo but little if any modification.

5. Bearing of these observations upon certain problems in the cytomorphosis of the mammalian erythrocyte:

a. From the preceding data it seems clearly evident that in contrast to the rather generally accepted view, the assumption of the definitive form of the erythrocyte is not necessarily initiated by or dependent upon an extrusion of the nucleus.

b. That on the contrary, a substantial body of facts supports the conclusion that correlated with the hemoglobin formation is the differentiation of a lipoid containing membrane which, as an important factor in producing a flattening of the cell, manifests itself in the pig embryo in the assumption of the definitive form of

the erythrocyte previous to the formation of the non-nucleated corpuscle.

c. An explanation of the assumption of an eccentric position on the part of the nucleus is suggested on the basis of the differentiation of a hemoglobin content tending to accumulate at one side of the cell and displacing the nucleus and the remainder of the original cytoplasm toward the opposite pole, a conclusion apparently supported by the character of the reaction of the erythroblast to osmotic changes and the occurrence in the circulation of free erythrocytic nuclei surrounded by a rim of cytoplasm, as indicative of structural differences between the nuclear and cytoplasmic poles of the cell.

2. *The origin of non-nucleated erythrocytes or plastids*

1. Structural characteristics:

a. In size the non-nucleated erythrocytes or plastids may vary from large plastids approximating the dimensions of the large erythroblasts, to smaller forms the size of blood platelets.

b. In form they vary from rounded or spherical to the definitive biconcave discs and concavo-convex or cup-shaped elements, the definitive forms predominating.

c. An occasional plastid may have a small, tapering cytoplasmic process projecting from its surface.

2. The origin of non-nucleated erythrocytes in blood cultures:

a. Autoplastic cultures were studied from pig embryos at a stage in which the formation of plastids in the circulating blood is at its height, but no conclusive evidence was obtained indicating the origin of plastids by the extrusion of the nucleus from the erythroblasts or of its disintegration within the cell.

b. On the contrary, a process was repeatedly observed and studied in which there occurred a constriction of the cytoplasm in the region between the nuclear and cytoplasmic poles of the erythroblast, which, when completed, resulted in the division of the cell into two parts, the one a nucleated structure, the other a non-nucleated hemoglobin containing corpuscle.

c. The erythrocytic nuclei remaining after the completion of such a cytoplasmic constriction may be surrounded by an appreciable quantity of cytoplasm, in other cases there may be little if any cytoplasm evident.

d. In size and hemoglobin content, these newly formed corpuscles are comparable to the normal plastids. As observed in the cultures, the majority when first formed were more or less spherical in shape, others manifest a tendency to become flattened, and some evidence was obtained in which a cup-shape was assumed while under observation in the cultures. An occasional plastid possessed a tapering cytoplasmic process which was observed to rise in connection with the final separation of the corpuscles from the parent cell.

e. A critical analysis of the technique, plasma, thermal and physical conditions in the cultures, together with the stage of the embryo, the normal and degenerative cytological changes and staining reactions of the erythrocytes to the end of ascertaining to what extent the above described behavior of the erythroblasts is to be regarded as normal or abnormal, indicates that the culture conditions are such as to justify anticipating some manifestation of the normal transitional stages in the origin of plastids.

3. Evidence concerning the origin of the plastids in the embryo by a similar process:

The occurrence in carefully fixed blood vessels of erythroblasts having a constriction of the cytoplasm between the nuclear and cytoplasmic poles, the variation in the size and form of the plastids, the presence of pyknotic erythrocytic nuclei surrounded by a rim of hemoglobin-containing cytoplasm, together with the observation in fresh vessels of the separation off of a cytoplasmic part of the erythroblast by a process of cell constriction, is evidence strongly indicating the origin normally of plastids in the embryo by a process of cytoplasmic constriction similar to that observed *in vitro*.

4. General considerations regarding the origin of non-nucleated erythrocytes:

a. Concerning the prevalent views of nuclear extrusion and intracellular disintegration, arguments so far as they are based

upon the indirect evidence from free erythrocytic nuclei in the circulation, erythrocytic nuclear inclusions in phagocytic cells, and nuclear particles in plastids, do not appear conclusive for either theory and seem equally well accounted for on the basis of cytoplasmic constriction. Nor is the more direct evidence for nuclear extrusion, at least in the embryo, sufficiently convincing but that the problem appears still to be an open one concerning which the present data for cytoplasmic constriction may be justifiably introduced.

b. Evidence from other mammals directly or indirectly supporting the present observations and conclusions is furnished, among others, by Malassez, Janosik, Engel and Howell. Similar evidence for the lower vertebrates by Giglio-Tos, Eisen, Engel and Jolly, appears of more than passing significance if the non-nucleated erythrocytes normally occurring in lower vertebrates are to be regarded as phylogenetically precursors of the respiratory elements which predominate in the adult animal.

c. The origin of non-nucleated erythrocytes by cytoplasmic constriction, if correct, renders the erythroblasts in this respect comparable with the megakaryocytes, plasma cells, and lymphocytes, to which they are apparently closely related genetically, for according to recent investigations these cells normally contribute to the mammalian circulation at least two important non-nucleated morphological elements other than plastids, by the constriction off of parts of their cytoplasm.

5. In conclusion, therefore, it may be stated that the data derived from the present investigation involving the study of blood cultures, living and fixed blood vessels in the pig embryo, together with the observations of other investigations for both red and white blood cells in various mammals, raises the question whether the origin of non-nucleated erythrocytes by a process of cytoplasmic constriction does not merit more serious consideration.

It is a pleasure to express here my appreciation of the encouragement received from Prof. R. J. Terry during the investigation and for valued criticism in the completion of this paper.

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DESCRIPTION OF FIGURES

Figures 1 to 12 a are from fixed blood vessels, figures 13 to 45 from fresh unfixed material and cultures. All the figures are camera lucida drawings. Figures 1 to 12 a were made with a No. 4 Zeiss compensating ocular and an oil immersion 2 mm. apochromatic objective, and figures 13 to 45 with a Leitz ocular No. 4, and No. 7 objective, the drawing-board being at the level of the table. The numerals at the right or left in figures 13 to 20 and 34 to 36, indicate respectively the time at which the observations were made.

ABBREVIATIONS

<i>e</i> , erythroblasts approximating the height of their differentiation prior to the formation of plastids	<i>nf</i> , erythrocytic nuclei with no evident surrounding cytoplasm
<i>em</i> , erythroblasts undergoing mitotic division	<i>p</i> , plastid
<i>eu</i> , undifferentiated erythroblasts	<i>pl</i> , large plastid
<i>ep</i> , partly differentiated erythroblasts	<i>pm</i> , medium-sized plastids
<i>n</i> , erythrocytic nuclei	<i>ps</i> , small plastids
	<i>w</i> , wall of blood vessel

PLATE 1

EXPLANATION OF FIGURES

1 to 12 a Erythrocytes at different stages of cytomorphosis. All drawings except figures 1, 2 and 9 a are made from blood corpuscles within the blood vessels in the foetal membranes surrounding the amniotic fluid of the pig embryo, carefully fixed in situ in the uteri, stained, cleared, and mounted in toto. All specimens were stained with Giemsa's fluid.

1 Young undifferentiated erythroblasts in the yolk sac of a 3 mm. embryo, showing large nuclei, spherical, basophilic cytoplasm, stained, cleared and mounted in toto.

2 Erythroblasts in a large sinus of the liver, as seen in section; 7 mm. embryo; *cp*, partly differentiated erythroblast showing a more or less biconvex or lens shape.

3 More highly differentiated erythroblasts, *e*, which have assumed biconvex and cup forms; 26 mm. embryo. Non-nucleated erythrocytes or plastids *pm* are beginning to appear in the circulation.

4 Mature erythroblasts and plastids of various sizes; 30 mm. embryo. The plastids now predominate numerically.

5 and 6 Erythroblasts *cc*, which appear to be giving rise to plastids by a process of cytoplasmic constriction, between the nuclear and cytoplasmic poles of the cell (compare with figures 13 to 18); 26 mm. embryo.

7 Erythroblasts with cytoplasmic process; 30 mm. embryo.

8 Plastids with cytoplasmic process; 30 mm. embryo. By mechanical rotation plastid *a* was demonstrated to be spherical in form.

9 Erythroblasts in the telophase of mitosis, showing a thread of cytoplasm, still connecting the daughter cells as suggestive of the mode of origin of cytoplasmic processes such as shown in figure 7; *a*, from vessel in foetal membrane of a 26 mm. embryo; *b*, from section of heart of 7 mm. embryo.

10 Group of corpuscles showing erythrocytic nuclei *n* with a narrow rim of hemoglobin-containing cytoplasm; 30 mm. embryo.

11 Group of corpuscles showing three erythrocytic nuclei, two of which *nf* correspond to the so-called 'free nuclei,' evidently deficient in surrounding cytoplasm; 30 mm. embryo.

12 Two erythrocytic nuclei *n*, surrounded by a considerable rim of cytoplasm. Note flattened shape of nuclei; 30 mm. embryo.

12 a Erythroblast containing two nuclei which have already assumed a cup shape; 30 mm. embryo.

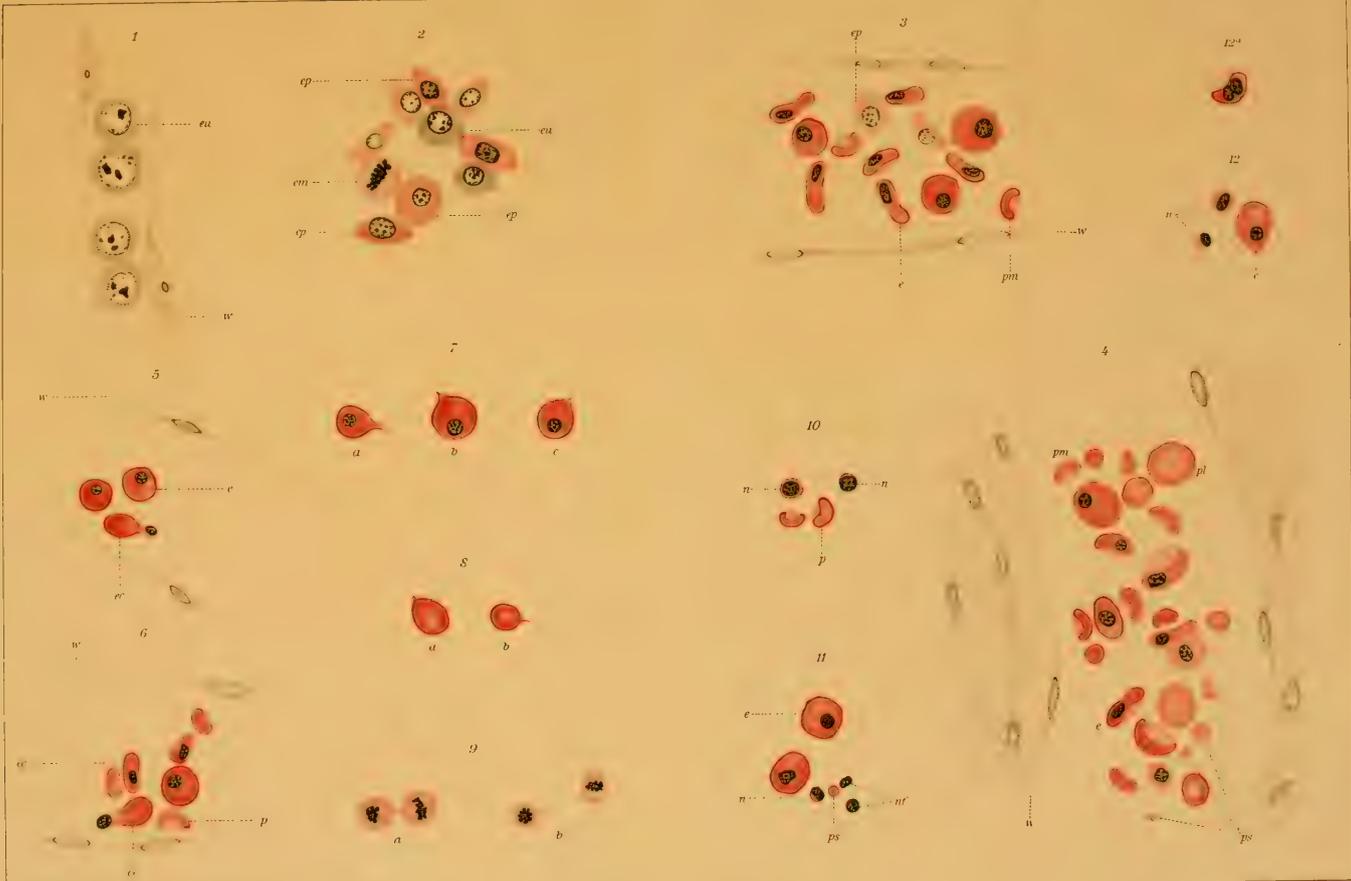


PLATE 2

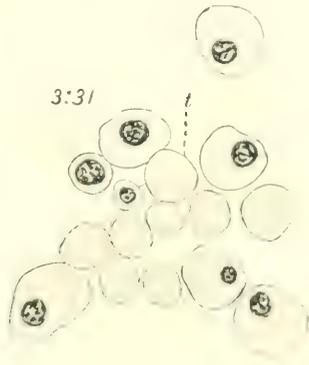
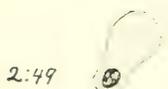
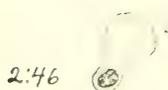
EXPLANATION OF FIGURES

13 to 14 Origin of plastids by cytoplasmic constriction.

13 Experiment 43,b; autoplasmic culture of the blood from the heart of a 30 mm. embryo. A series of stages showing the formation of a plastid by the constriction off of the cytoplasm of an erythroblast. Examined immediately after the preparation was made. In stage *t* the surrounding corpuscles are included for comparison; when the last drawing was made it was supposed that the constriction process had been completed and the plastid entirely detached, but closer observation made evident an almost invisible thread still connecting the two parts, the continuity of which was still further tested by a mechanical disturbance of the cells. It may be noted that there was no cytological difference evident between this newly formed non-nucleated element and the neighboring plastids. Compare the nucleated remainder with the erythrocytic nuclei *n* in figures 10, 11 and 12.

14 Experiment 49; forty-three-hour autoplasmic culture from a 27 mm. embryo. Origin of medium-sized plastid from erythroblast by constriction process.

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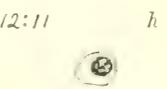
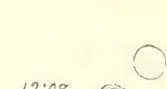
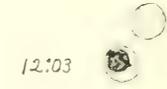
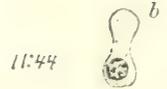
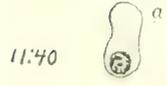


PLATE 3

EXPLANATION OF FIGURES

15 to 18 Origin of plastids by cytoplasmic constriction.

15 Experiment 20; nineteen-hour autoplasmic culture; 30 mm. embryo. Formation of large plastid.

16 Experiment 21; nineteen-hour homoplasmic culture of blood from 30 mm. embryo in plasma, from 190 mm. embryo. Formation of large plastid, comparable to the so-called macrocytes.

17 Experiment 20; twenty-four autoplasmic culture; 30 mm. embryo. Shows the partial fragmentation of the cytoplasm remaining around the nucleus (stages *r, s*) after the completion of the cytoplasmic constriction and the origin of a small, secondary plastid or microcyte from the newly formed plastid (stages *l-s*).

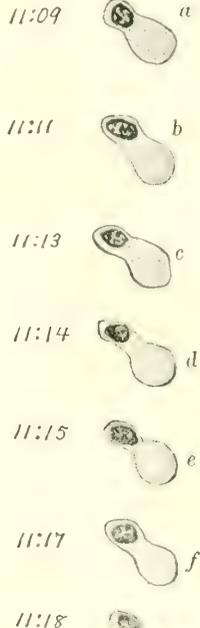
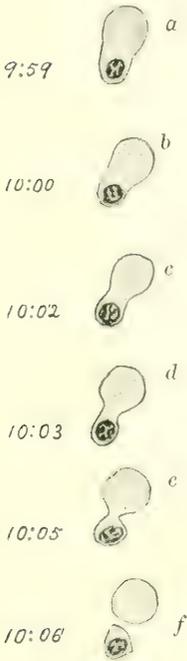
18 Experiment 9; approximately a twenty-hour homoplasmic culture of blood from a 32 mm. embryo, in plasma from a 49 mm. embryo. Shows origin of a plastid by a cytoplasmic constriction in which the process was of such a character as to leave the nucleus unsurrounded by any evident cytoplasm.

ERYTHROBLASTS IN THE PIG EMBRYO

VICTOR E. EMMEL

PLATE 3

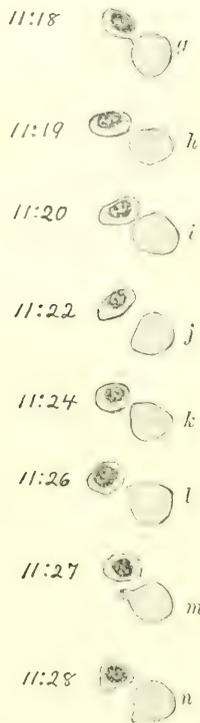
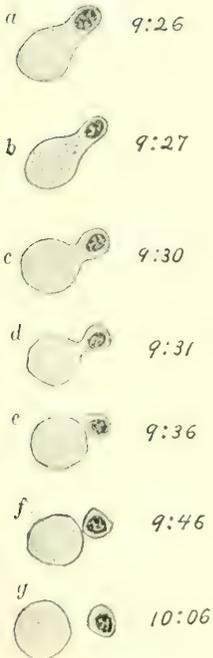
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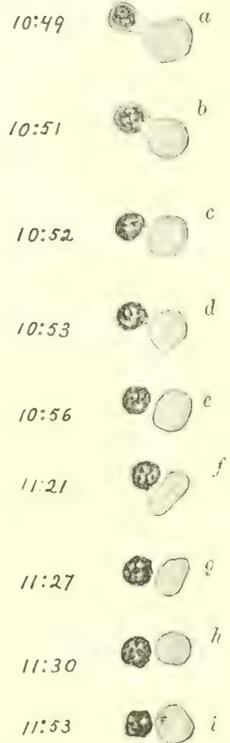


PLATE 4

EXPLANATION OF FIGURES

19 to 31 Origin of plastids. Cytological characteristics of erythrocytes.

19 Experiment 3; twenty-one-hour homoplastic culture of blood from a 28 mm. embryo in plasma from a 52 mm. embryo. A constriction rather irregular in character, showing the origin of three small plastids (stage *f*), one of which while under observation was seen to assume a cup shape (stages *g-n*). In this instance the plastid was undergoing a slow rotation, presenting various views as shown in stages *m* and *n*.

20 Experiment 9; nineteen-hour homoplastic culture of the blood from a 32 mm. embryo in plasma from a 49 mm. embryo. Shows a somewhat imperfect case of constriction in which the cytoplasm between the nucleus and the forming plastid became drawn out in a slender thread before the plastid became detached.

21 Erythroblast from heart of 35 mm. embryo. Beginning at the left, *a*, *b*, *c*, and *d* are successive aspects presented during a single rotation of the cell as it was mechanically turned over while under observation, showing the tendency of the nuclear pole to swing undermost in response to gravitation. The profile view *c* also shows the biconcave disc form.

22 Erythroblast from 28 mm. embryo, after having been kept two days in Ringer's solution. Shows an unequal response of the nuclear and cytoplasmic poles to osmotic changes; *b* is the profile view of *a*.

23 and 24 Erythroblasts which, although containing two nuclei, have already assumed cup and flattened disc forms, respectively; from a 25 mm. embryo. In each case *b* is a profile view of *a*.

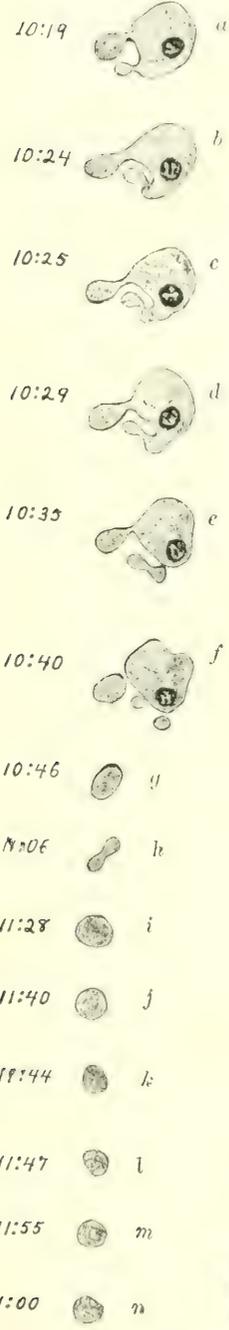
25 Face *a* and profile *b* views of an erythroblast showing a constriction in the region between the cytoplasmic and nuclear poles. From a culture of the blood of a 32 mm. embryo in the plasma of a 65 mm. embryo. Experiment 60.

26 Face *a* and profile *b* views of a small plastid showing biconcave disc form; 25 mm. embryo.

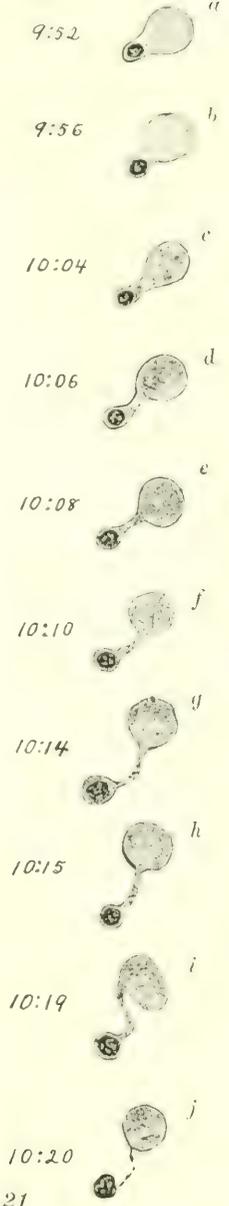
27 and 28 Small and medium sized plastids which by mechanical rotation were demonstrated to be spherical in shape; 28 mm. embryo.

29 to 31 Medium sized and large plastids showing cup and disc shapes. Each figure represents successive views of a mechanically rotated corpuscle, cf. fig. 19.

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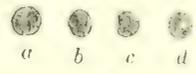
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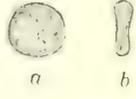


PLATE 5

EXPLANATION OF FIGURES

32 to 45 Cytoplasmic constriction in living vessels. Cytological characteristics and degenerative changes in cultures.

32 and 33 Erythroblasts as observed within the living vessels in the foetal membranes of a 22 mm. embryo, immediately after the removal from the uterus. Shows cytoplasmic constriction and origin of a non-nucleated element in a manner comparable to the process observed in the cultures (figs. 13-18). Neighboring erythrocytes in the vessel not drawn.

34 Experiment 4; twenty-five-hour autoplasmic culture, + Ringer's solution, of the blood from a 25 mm. embryo. Shows the origin and behavior of vacuoles, presumably degenerative in character. At stage *f* the original vacuole has become divided into two parts.

35 Experiment 52; migratory movements of a small vacuole in the cytoplasm of an erythroblast; sixty-nine-hour autoplasmic culture of blood from a 33 mm. embryo.

36 Amoeboid-like changes in the cytoplasm of an erythroblast as observed in the living blood vessels in the foetal membranes of a 28 mm. embryo immediately after the removal from the uterus.

37 to 40 Vacuoles in the cytoplasm of erythrocytes showing a tendency to collect around the nucleus in the erythroblast (figs. 38-39) and at the periphery of a plastid (fig. 40). Experiment 54; twenty-hour autoplasmic culture from a 25 mm. embryo.

41 Erythroblast in fresh blood just taken from the heart of a 27 mm. embryo, showing a thickening of the cytoplasmic rim of the disc in the region of the nucleus.

42 Experiment 51; twenty-one-hour autoplasmic culture from the blood of a 30 mm. embryo. Erythroblast in telophase of mitosis, the daughter cells being still connected by a thread of cytoplasm (compare with figure 7).

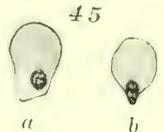
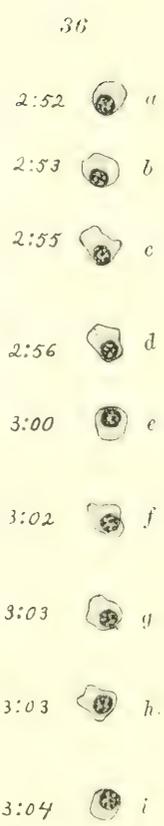
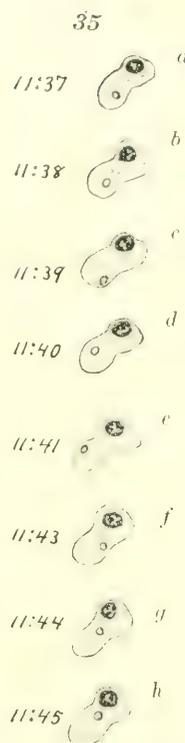
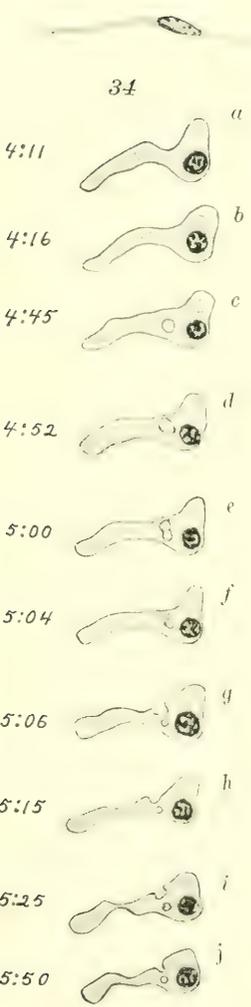
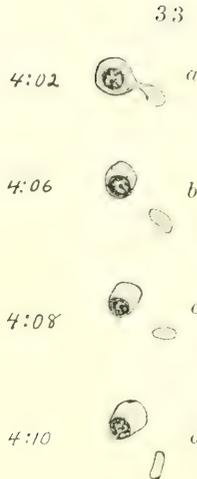
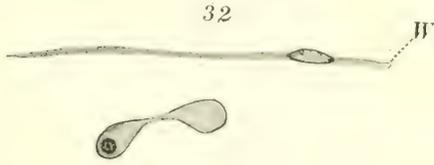
43 and 44 Cup-shaped erythroblasts; Experiment 55; two-day autoplasmic culture from the blood of a 31 mm. embryo.

45 Experiment 52; four-day autoplasmic culture from the blood of a 33 mm. embryo. Two aspects of an erythroblast, in the profile view *b* of which, the nucleus presents the deceptive appearance of a nuclear extrusion.

ERYTHROBLASTS IN THE PIG EMBRYO

VICTOR E. EMMEL

PLATE 5



THE STRUCTURAL UNIT AND GROWTH OF THE PANCREAS OF THE PIG¹

GEORGE W. CORNER

Assistant in Anatomy, Johns Hopkins University

NINETEEN FIGURES

The conception of the formation of the body from a series of repeated units, has been studied along three main lines. The first, that which has to do with gross anatomy, early became a special science, transcendental anatomy; and the vertebra as a unit of bodily organization was carried to fantastic lengths by Oken and Goethe. With the work of Schleiden and Schwann, and the progress in microscopy, attention was called to the cell as the unit of structure. The third, and latest, aspect of the subject lies in the borderland between gross and microscopic anatomy. Since the discovery of the liver-lobule by Wepfer in 1664, evidence has been accumulating to show that many organs of the body are formed from small masses of tissue, which, regularly repeated, compose the whole organ. The first grosser divisions of organs, the lobes, were made out by the earlier anatomists. When fine dissections, and later the microscope, unveiled more minute divisions in glands, the word 'lobulus,' diminutive of 'lobus,' entered the vocabulary. In the course of time, the word has come to have a double meaning. With some, 'lobule' is, as with the older observers, merely a vague descriptive term applying to a small mass of tissue limited by connective tissue; with others, it is a term of accurate scientific connotation, so that arguments have arisen as to whether this or that part of an organ is 'the lobule.'

This is the case in writings upon the pancreas, to which the word seems first to have been applied by Albrecht von Haller,

¹ Aided by the Carnegie Institution of Washington.

for I do not find it in any antecedent work, nor in a painstaking review of current knowledge of the pancreas published in an inaugural dissertation by one Philip D'Orville at Leyden in 1745, nineteen years before the celebrated *Elementa Physiologiae* appeared. Haller's words are these: "Totum nempe sit ex lobis, parum distinctis, qui & ipsi ex minoribus fiunt *lobulis*, iique minores lobuli & ipsi in acinos discedunt." (1764.)

In later times Harris and Gow ('93) in describing the pancreas as found in a large number of species, apply the term lobule in the old variable sense:

The size and shape of the lobules of the gland vary considerably in the glands of different animals and indeed to a lesser extent in the same gland, but the general characteristics are fairly well maintained in each gland. In the human pancreas, for example, the lobules are large and irregular in shape, but they are distinctly mapped out from each other; in the monkey they are larger but less distinct; whereas in the pancreases of the cat and dog and other animals closely associated with them the lobules are small and quite distinct; in the dingo-dog, glutton, and weasel the lobules are still smaller; on the other hand in certain pancreases hardly any differentiation can be made out, and the tissue appears almost exactly like sections of the liver, or still more like sections of the adrenal.

Opie ('03) and Flint ('03) took the cat's pancreas as the type, and described as the lobule the smallest portion of the gland which is separated off by connective tissue. According to their descriptions this 'primary lobule' is a rounded mass from 1 to 2.5 millimeters in diameter, surrounded by connective tissue, drained by a duct, and supplied either by an arteriole or by capillaries from an interlobar artery. From six to twenty of these primary lobules are grouped together to form a 'secondary lobule,' which is provided in turn with its own artery and duct, composed by the union of the vessels of the component primary lobules. Laguesse ('05) does not consider the primary lobule of Opie an independent unit. (He worked with human material, in which, as pointed out by Harris and Gow, the demarcations are not similar to those in the cat.) He believes the secondary lobules, or lobule-groups, of Opie to be the smallest indivisible regularly repeated units composing the pancreas, and complicates the situation by giving the term 'lobule' to the larger

structure, calling the lobule of Opie 'lobulin.' Lobule of Opie = lobulin of Laguesse; secondary lobule, or lobule-groups, Opie = lobule, Laguesse.

It is more than a mere question of descriptive terminology, for recent researches have shown that there is a fundamental significance underlying the construction of organs from small units. The villus of the intestine, long known, was demonstrated by Mall in 1887 to be a unit of structure; the unit of the spleen was demonstrated by the same investigator in 1900; of the lung by Miller ('92); of the salivary glands by Flint ('04). In 1906, Mall applying the ideas of Thoma regarding the capillary bed to a study of the liver, summed up and greatly extended our idea in this direction. He showed that the organ is composed by the repetition, thousands of times, of a definite unit, that the whole liver at its earliest stage in the embryo is simply one of these units; that in the adult animal of many species the outline of the unit may be masked by the growth of connective tissue radiating from the portal vein, so that what is commonly known as the liver-lobule is not the structural unit; and that certain definite relations hold between the size of the unit and the length of capillary blood vessels.

From all this work the variable quantity 'lobule' has given way to the definite conception 'structural unit.' The use of this term was urged by Minot in his presidential address of 1904, the definition then given being: "The territory of an organ supplied by a single terminal branch of an afferent vessel (artery or vein)." The same entity can now be better defined from a somewhat different point of view: The structural unit of an organ is the smallest part of it which is regularly repeated in a similar way throughout, and which contains the elemental constituents of the organ under consideration; that is to say (if it is a gland of external secretion) gland substance, duct, and blood-vessels. Now for the organs thus far studied, the structural unit thus defined proves to fulfil Minot's definition as well. The length of a blood-capillary determines the size of the unit. In the liver the unit is as large as the capillary bed of a terminal portal vein (Mall '06). In the spleen (Mall '98, '00) the capil-

lary bed of a terminal arteriole measures the unit. The villus of the intestine is supplied by a terminal arteriole; and instances might be multiplied to show that it is the circulatory system which in this way lays off the organ into units. Moreover, the division is one of almost mathematical exactness. The investigations of C. Ludwig and his pupils have shown that all the capillaries connecting the terminal tips of the arteries and veins are of the same length in any one organ. Then Thoma ('76) has made clear that all the capillaries of any one organ are equally favored with respect to pressure and rate of flow by their very laws of growth; and Mall, as we have seen, extended this idea by showing that all the units of an organ (the liver) are equally favored with respect to the circulation.

To summarize briefly: Those subdivisions of glandular organs which we discover by the eye, dissection and the microscope, are not random; these organs are composed of regularly repeated units, each complete in itself as to structure and function, whose size is determined by the length of blood-capillaries. Such units appear to persist throughout the development and adult state of the organ; and if for some special reason the unit is concealed by over- or under-growth of the limiting connective tissue at one stage of ontogeny, it may still be traced at another.

We have seen that there is much confusion regarding the subdivisions of the pancreas and their significance. The following study is undertaken, therefore, to determine the structural unit and method of growth of the pancreas by careful investigation of a single species, in the light of the general conception outlined in the foregoing pages, and to make whatever addition may be possible to the general theory.

TECHNIQUE

The pancreas of the pig was taken as the object for study because of the ease of obtaining embryonic material in all stages, as well as adult. As the new observations reported in this paper, as far as the development is concerned, depend upon a method of injecting the ducts of the embryonic and foetal pancreas, I shall describe the technique in some detail.

E. C. Hill ('06) working in this laboratory, first injected the embryonic bile-ducts in pigs by filling the stomach with India ink by a hypodermic syringe. He attempted the same experiment with the pancreas, but merely reports "not very fruitful results." I have followed this hint and developed a successful method. To inject smaller embryos, hollow glass needles are used, which must be drawn from tubing, of such a sharpness that they will easily pierce, without tearing, the paper-thin walls of the stomach; at the same time the needles should taper quite rapidly, so as to plug tightly the aperture made by them in the stomach, and thus prevent regurgitation of the injection-fluid. The operator holds in his mouth a rubber tube, reaching to his hand; at the end of this tube the needle is inserted (Popoff's method, '94). The needle is filled by suction with the fluid, which is Higgins' waterproof ink, diluted. The embryos need not be warm, but should be used within 2 to 3 hours after removal. The left abdominal wall is slit open, the stomach exposed, and the needle thrust into its lumen. The operator then blows forcibly until the ink has distended several coils of intestine. The stomach acts as a pressure bag, and the ink usually backs up into the pancreatic ducts without extravasation. The extent of the injection cannot be controlled; it may be partial, complete in the head of the pancreas, or quite complete.

This method succeeds only in embryos from 30 to 70 mm. long. Below 30 mm. the ink does not enter the pancreatic duct, although it may easily be forced through the entire intestinal canal. Above 70 mm. the diagonal course of the duct through the intestinal wall acts as a valve to prevent influx of the ink. In the larger embryos and foetuses, therefore, recourse must be had to still finer methods in order to enter the duct itself. I have had full success with the method used in this laboratory for some years by several investigators in the study of blood and lymphatic capillaries. Delicate hollow glass needles are drawn, of a diameter of 25 to 50 micra. The intestinal viscera are removed from the embryo and very carefully dissected under the binocular microscope in water or salt solution until only the stomach, duodenum, and pancreas re-

main. The duct is seen as a translucent streak passing down to the intestine about in the center of the duodenal portion of the head (figs. 12, 13). When it is located the needle is thrust into it just where it emerges from the intestinal wall, and the ink is blown in through a rubber tube held in the operator's mouth. The progress of the injection can be watched and controlled, but the method requires much more dexterity than the other. In foetuses from 70 to 80 mm. the operation is quite a difficult one, for the duct is very small, not easily distinguished, and yields before the needle. With practice, however, a complete series of preparations can be obtained by these two methods. The specimens are preserved in 10 per cent formalin, dehydrated, and cleared by Spalteholz's or other clearing fluids.

The blood-vessels are filled with ink, Berlin blue, or silver nitrate solution through the aorta or coeliac axis, according to the size of the embryo. Double injections of embryonic ducts and blood-vessels are easily made, but prove confusing through complexity. I have used the ordinary simple methods of injecting the adult specimens, the tissue being cleared en masse or cut into serial sections.

PANCREAS OF 100 TO 200 MM. PIG

The best point at which to begin the study of the structural unit of the pancreas is in the foetus of 100 to 200 mm. length (figs. 1, 2). At this stage the organ is relatively small and simple, and the duct and blood-vascular systems are injected without difficulty. In form and relations the gland is closely similar to that of the adult. At the 120 mm. stage the head lies close to the duodenum for a distance of 4 or 5 mm.; the duct enters the duodenum at the lowest point of the head. The tail stretches toward the spleen, and is joined to the head by two narrow bridges of gland tissue, the upper one of which (fig. 1, *UB*) represents the dorsal anlage, the lower (fig. 1, *LB*) the ventral anlage, according to Thyng ('08) (fig. 2, *B*). Where the lower bridge joins the tail there is a broad thick descending process which fits neatly into the upward curve of the duodenum (fig. 1, *Pr.*). Minor variations from this form will be seen in many of

the figures. The tail of the pancreas is supplied with blood by one or more arteries from the splenic branch of the coeliac axis; the head is supplied by the superior pancreatico-duodenal branch of the coeliac axis; and the descending process is partly fed by the inferior pancreatico-duodenal from the superior mesenteric trunk. There is normally one duct, since in the pig the duct of the dorsal pancreas alone persists (Stoss '91; Wlassow '95; Thyng '08)

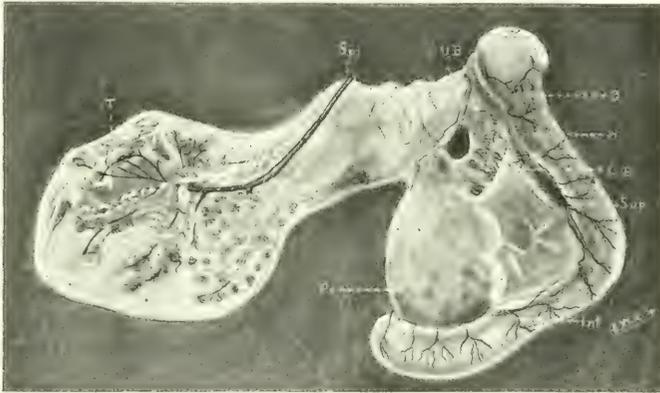


Fig. 1 Pancreas and duodenum of a 120 mm. pig foetus, dorsal aspect. Arterial and capillary blood-vessels injected with ink from aorta. *D*, duodenum; *H*, head of pancreas; *T*, tail of pancreas, showing lobulation; *UB*, 'upper bridge;' *LB*, 'lower bridge.' $\times 4\frac{1}{2}$.

As shown in the figures (2, 13) it sends branches to all parts of the gland. It is clearly seen from the diagram (fig. 2, *A*) that since the duct and blood-vessels enter the organ at different points, there is no constant relation between the branches of the duct and the arteries. In one part of the pancreas the two systems run parallel, in another they may course in opposite directions (see below, p. 233).

The secretory tissue is not homogeneous, but is divided off into small rounded masses, which are clearly seen with the naked eye, in the tail of the pancreas, standing apart like a branch of grapes in the jelly-like mesenchymal tissue (fig. 1). Toward the head they are condensed and crowded together, but may be

separated by teasing. When these nodules are dissected out and studied under the microscope it is found that they are mostly about 0.4 to 0.7 mm. in diameter; some are larger, and some smaller, but the larger masses are greatly lobated, as if in the

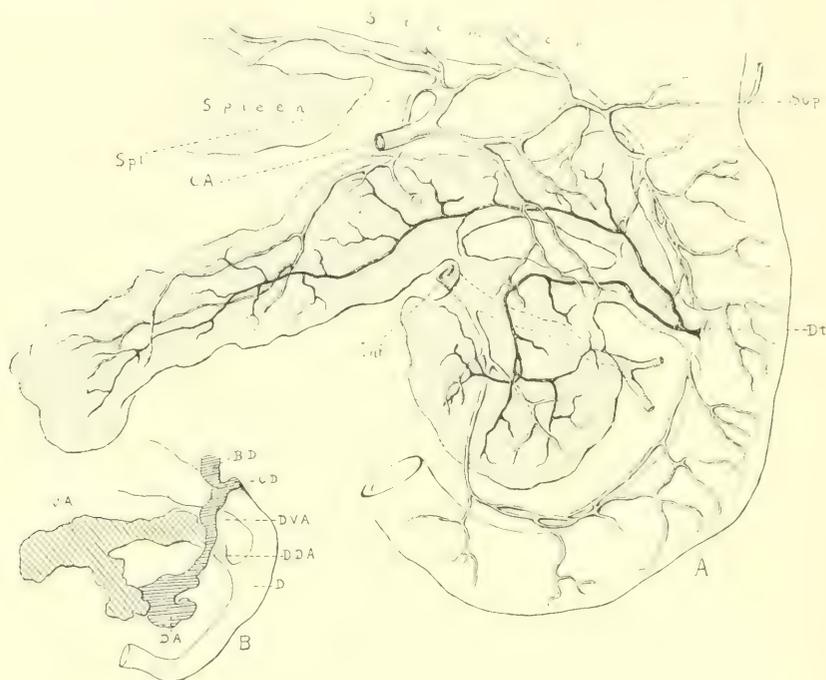


Fig. 2, A Diagram showing pancreas and duodenum (dorsal view) of 150 mm. pig foetus in which both arteries and ducts have been injected; to illustrate the point that the courses of the ducts and arteries are parallel in some part of the pancreas but not related in others. *D*, duodenum; *CA*, coeliac axis; *Spl*, branch of splenic artery supplying pancreas; *sup*, sup. pancreatico-duodenal artery; *Inf*, inf. pancreatico-duodenal (from superior mesenteric); *Dt*, duct.

Fig. 2, B Duodenum and pancreas of 20 mm. pig embryo, dorsal view (after Thyng '08, slightly altered) showing the part taken by the two anlagen in the formation of the pancreas. *D*, duodenum; *VA*, (light shading) ventral anlage; *DA*, (dark shading) dorsal anlage; *CD*, common duct; *BD*, bile duct; *DVA*, duct of ventral anlage; *DDA*, duct of dorsal anlage (main duct in the pig).

act of dividing, and the smaller ones are usually found in close groups or intimately joined to larger nodules. From this fact, and from other points to be mentioned later, it is fair to assume

for the present that the smoothly rounded average-sized nodules represent typical divisions of the foetal gland, while the larger and smaller masses are stages in the multiplication of one mass into two or more. From now on, for the sake of clearness, these bodies will be referred to as 'structural units,' since one of the results of this study is to prove them such.

In injected specimens a branch of the duct can be traced into each unit (fig. 16) where it branches by dichotomous division a number of times to reach the acini. The first or intralobular

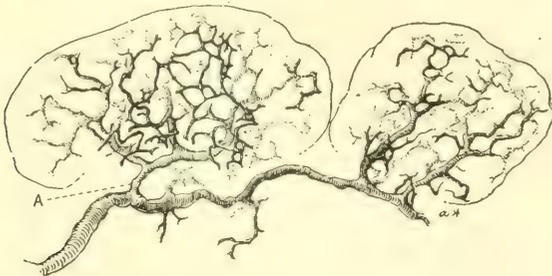


Fig. 3 Part of pancreas of a 105 mm. pig foetus. Blood-vessels injected with silver nitrate, showing arterioles and capillary tufts of two units; one is supplied by an arteriole which branches before entering the capillaries, the other has two tiny arterioles. Preparation by Dr. Florence R. Sabin. A, arteriole supplying one unit. $\times 55$.

branches are several in number, tending toward all parts of the unit, and give off slender straight ducts (fig. 16) corresponding to the 'Schaltstücke' of the adult; each of which in turn gives origin to a few slender short pre-acinar ducts, whose ultimate twigs are the acini. In figure 13, B, the artist has shown the duct-system in its full complexity. An arteriole penetrates into each unit (fig. 1) where it may branch once into the tiniest of arterial vessels, and immediately breaks up into a ball-like capillary net of great richness (fig. 3, A). This capillary net may anastomose with a few or with many capillaries from adjoining similar nets, according to whether it is situated in the denser body of the gland, or is lying relatively free in the tail. A cleared arterial injection presents a beautiful appearance under the binocular microscope, being a thick cluster of these capillary

balls. The capillaries are so numerous that every acinus is encircled by a mesh of the net at intervals of 15 or 20 micra. There is no regular relation, within the unit, between the duct and the blood-vessels. As mentioned before, according to the position of the unit with respect to the nearest duct and artery, its ductlet and arteriole may enter it at adjacent, or at opposite points (fig. 17, A). The duct to a single unit has a diameter of about 0.015 to 0.020 mm.; and the artery measures about 0.0125 mm. in diameter.

THE ADULT PANCREAS

The fresh pancreas of the pig presents an irregular surface which gives at first the impression of being divided into lobes. However, if one take a blunt probe and separate the areolar tissue between these lobes, all semblance of regularity in them is lost, as the dissection divides the organ into an increasing number of parts. But when the dissection has proceeded so far that the probe can go no farther without tearing glandular tissue, it is found the organ has been divided into a large number of small masses of surprisingly regular size. These masses, which as found in the pig's pancreas agree with the secondary lobules or lobule-groups described by Opie (and themselves called lobules by Laguesse), are of somewhat varying shape, generally rounded, wedge-shaped, or roughly pyramidal. They average about 0.025 grams in weight (formalin specimen washed in tap-water and dried on filter-paper); and as the average weight of five entire adult pancreases dissected cleanly and weighed under similar conditions was 41 grams, an adult pancreas of normal size is composed of about 1000 lobule-groups. In injected specimens one artery is found by dissection to enter each of these lobule-groups, which in turn is drained by one duct. The average diameter of the artery is 0.136 mm., the duct 0.093 mm. The ducts from lobule-groups unite in tree-like form, becoming at last lateral branches of the main-duct, entering it at right-angles all along its course. The grosser pancreatic ducts in the pig sufficiently resemble those of the dog, illustrated by Revell with beautiful corrosions ('02) to render further description unnecessary here.

If the lobule-group is sectioned, it is seen to be itself divided off into smaller divisions by fine septa of areolar tissue. As Harris and Gow found, the division is far from constant. It varies greatly in different parts of the same gland, and even what seems to be a large undivided area is found on tracing down the serial sections to become divided into lobules. The strands of the connective tissue are sufficiently marked in most parts of the pancreas to permit reconstructions from the sections. Such reconstructions show the lobule-groups to be composed of twenty

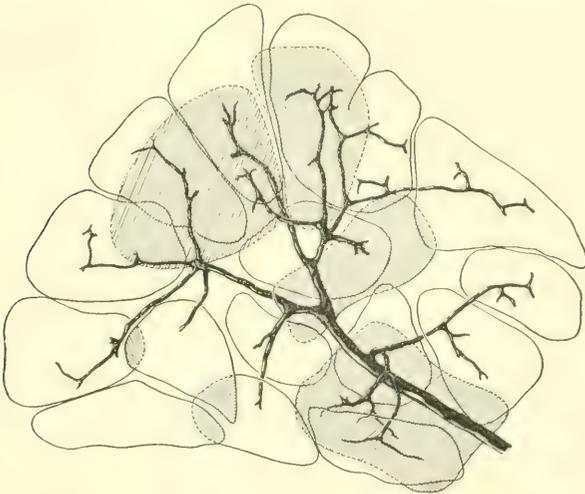


Fig. 4 One lobule-group of adult pancreas, duct-injection. Reconstructed from serial sections. $\times 25$.

to thirty rounded or wedge-shaped masses from 0.5 to 1.0 mm. in diameter (fig. 4). These lobules are usually united to each other, so that adjoining lobules often run together at their bases, and most of them cannot be clearly distinguished in the pig's pancreas without tracing them through the series. For intelligent study of the duct and blood-vascular systems it is necessary to make thick slices of injected specimens, and in these preparations it is only by chance that one finds a lobule standing out

clearly from its neighbors. When we do find such a specimen of the structural unit, it is entered by one artery (fig. 5, *A.A.*), but has in addition many capillary anastomoses about its periphery with the capillaries of the neighboring lobules. There is usually a chief venule (fig. 5, *V.V.*) which may or may not run along with the artery. In addition, there are often one or two other small veins connecting with the capillary net.

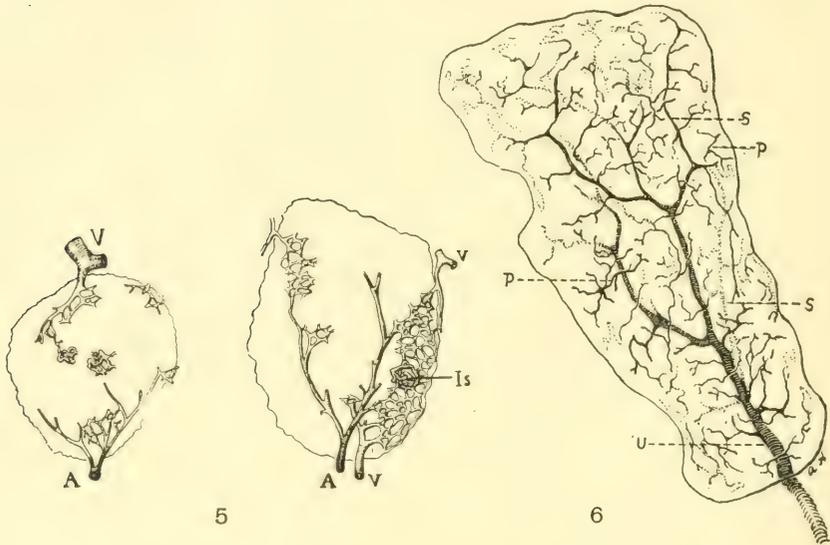


Fig. 5 Diagrams of two units of the adult pancreas, drawn with the camera lucida from thick sections of a specimen in which the blood-vessels have been injected. *AA*, arterioles supplying units; *VV*, venules draining units; *Is*, dilated capillaries of an island of Langerhans. $\times 20$.

Fig. 6 Cleared injection of adult pancreas, one unit dissected out, showing ducts. *U*, unit-duct; *S*, straight ducts; *P*, pre-acinar ducts. $\times 40$.

Dogiel twenty years ago ('93) studied the ducts of the pancreas in sections of Cajal silver specimens, and described four orders, as follows: (1) Large excretory ducts; (2) tubules of the second order; (3) tubules of the third order, or "Schaltstücke;" (4) very fine tubules of the fourth order, which often divide again and finally enter the acini.

Of these orders he did not relate the first two to the architectural landmarks; probably they are not anatomical entities. The third and fourth orders of Dogiel, however, have a definite meaning. (Stöhr gives practically the same thing in his well-known textbook ('06).) By finding a clearly demarcated unit in a thick section of the pancreas injected through the duct, or by carefully dissecting out one unit with fine needles, we can easily relate the ducts of various sizes to the structures of the gland (fig. 6). Exactly as in the embryonic tissue, the single efferent duct of the unit has four or five branches. These are much less regular than the intralobular ducts of the salivary glands, and hence should not, perhaps, be dignified with a special name. They scatter to the various parts of the unit, where each branches two or three times to give off the straight ducts (3rd order of Dogiel) or *Schaltstücke* (fig. 6, *s*); these give rise to the very tenuous twig-like preacinar ducts (fig. 6, *p*) whose branches are the acini themselves. The acini seem to be terminal vessels. Ramón y Cajal and Sala ('91; quoted in Oppel '00, T. 3, p. 796) state, from the study of silver-impregnated specimens, that there are no anastomoses between the terminal acini in the frog, hake, chicken, hedge-hog, guinea-pig, or rabbit. Renaut ('99) says there are anastomoses at the periphery of the lobule in birds, not in the human. My specimens show no anastomoses in the adult pig, but perhaps ink-injections should not be accepted as conclusive evidence upon this point. The question is of interest in connection with the embryonic stages to be described below.

The ducts can be numbered and listed, upon the basis of the foregoing description, as follows: (1) Main duct of the pancreas (Santorini); (2) the laterals and their branches; (3) ducts to lobule-groups, about 1000, all told; (4) unit-ducts, about 20,000 to 30,000; (5) the straight ducts; (6) pre-acinar ducts; (7) the acini. But names and numbers mean nothing except so far as they relate the vessels to the subdivisions of the gland.

I have already pointed out that there is no parallelism between the artery and duct in the embryo (fig. 2). Yet even though the two vessels enter directly opposite sides of the embryonic lobule, by the time that same lobule has grown to be an extensive tree

in the adult, the two vascular systems may be running side by side at some points, and therefore in the adult the lack of relation will be less apparent than in the embryo (fig. 17). Still, it is in many places striking, although in other instances a duct and artery are often closely parallel. This explains perhaps, why some observers describe the pancreatic vessels and ducts as running together, others as separate systems.

Flint ('03, '04) has shown that in the cat and man this elaborate structure of acini, ducts, and blood-vessels is supported by a delicate reticulum of connective tissue, which is slightly condensed at the margin of the lobule to form the thin interlobular septum ('04, fig. 8, p. 98). The islands of Langerhans are also surrounded by a slightly condensed stroma. In the pig I find the number of islands in one unit a variable quantity (none, one, two, three, or more). In this paper I have considered the exocrine portion of the pancreas, omitting detailed studies of the islands. Bensley ('11) has given a full account of the structural relations of the islands of Langerhans in another species (the guinea-pig).

We have thus demonstrated a circumscribed portion of the pig's pancreas, which is regular in size and form, and retains its characteristics from foetal life to the adult stage, except that its boundaries are indistinct in the adult. This unit is a miniature pancreas, for it has secretory tissue, a supporting framework of connective tissue; an artery, capillaries, a vein; and a duct; and yet is itself divisible only into dissimilar fragments. In the foetus, where the pancreatic tissue has ample space and is not compressed, the unit has typically the form of a sphere whose radius is as long as a blood-capillary. The whole adult pancreas is made up of twenty or thirty thousand units of this kind, repeated side by side. The unit agrees closely with that described by Opie in the cat. From a few observations the lobulation of the guinea-pig's pancreas seems also to be the same as that of the domestic pig; whereas in the adult dog and human the outline of the units is lost.

GROWTH OF THE PANCREAS

The earliest duct-injection figured in this paper is from an embryo measuring 35 mm. (fig. 7). Although the whole organ is not injected, the ducts in the head seem to be completely filled. The picture is an unexpected one. There is no sign of



Fig. 7 Pancreas and duodenum of a pig foetus 35 mm. long, ventral view. The ducts in the head have been injected by forcing ink from the stomach (p. 211) and appear as a capillary plexus without a main channel (it is not necessary to ligate the intestine in making these injections). *D*, duodenum; *E*, entrance of duct into duodenum; *Pl*, plexus of ducts. $\times 30$.

a main duct with branching arms, but instead the tubules form a plexus, with frequent anastomoses at fairly regular intervals. This plexus is connected with the intestine by a narrow tubule

no more capacious than any other strand of the net. A few blind twigs project at different points; some of these are perhaps indicative of incomplete injection, but others, no doubt, are the beginnings of new meshes.

There are a few hints of this early stage of the pancreas in the literature. Hill ('06) found that the early bile-ducts are plexiform. Some of his preparations are illustrated in Mall's study of the liver unit ('06). Laguesse ('95-'96) has made a careful study of the early growth of the sheep's pancreas by sections and graphic reconstructions. According to him, the anlagen grow out as solid cords, which anastomose "like the cords of Remak in the liver." When the embryo is 18 mm. long, all these cords have become hollowed out, constituting the "primitive pancreatic tubules." No doubt injections of this and the immediately succeeding stages would give pictures identical with that here figured in the pig of 35 mm. Next, according to Laguesse, the progenitors of the true secreting tubules grow out as buds from the sides of the primitive anastomosing tubules, and the anastomoses disappear slowly, but by searching may be found in the sheep embryo of 115 mm. These statements were verified by Renaut ('99); and Völker ('02) found the earliest tubules of the pig's pancreas to anastomose. The injection method now enables us to give positive proof of these observations, and to follow the subsequent changes with ease.

Thus, in the pig of 40 mm. (fig. 8, *Dt*) there is seen the first sign of a main excretory duct amid the plexus. One faintly distinguishes a slight increase in the diameter of some of the tubules in the head of the pancreas, as if a channel were forming. The formation is complete in the 50 and 60 mm. specimens of this series (figs. 9, 10). Here there is a definite excretory duct, which runs through the head of the pancreas to the duodenum in the course taken by the duct in the adult. This channel gives off a great number of branches, which all anastomose, so that the pancreas is composed of a close net of capillary ducts, with a channel coursing through it to the intestine. The resemblance to an artery and its capillary net is complete; and no one who has seen Thoma's figures of the primitive blood-plexus can fail

to be struck by the similarity of the process here described to the growth of an artery from the primitive plexus (Thoma '76). The suggestion immediately occurs that the pancreatic duct appears, like an artery, because there is a hydrostatic necessity for it; in other words, because fluid is passing through the plexus,



Fig. 8 Pancreas and duodenum of a pig foetus 40 mm. long, ventral view. Partial injection of ducts, from stomach. First suggestion of a duct-channel running through the capillary net. *D*, duodenum; *Py*, pylorus; *E*, entrance of duct into duodenum; *Dt*, beginning formation of duct-channel. $\times 17$.

and demands a passage way. There is some slight evidence in favor of this hypothesis; the new channel becomes larger and larger toward its outlet, as more and more capillaries enter it; again, the pancreatic cells contain zymogen granules as early as 20 mm., as shown by examining the fresh tissue in salt solution. Both these facts hint at the presence of a secretion, and there-

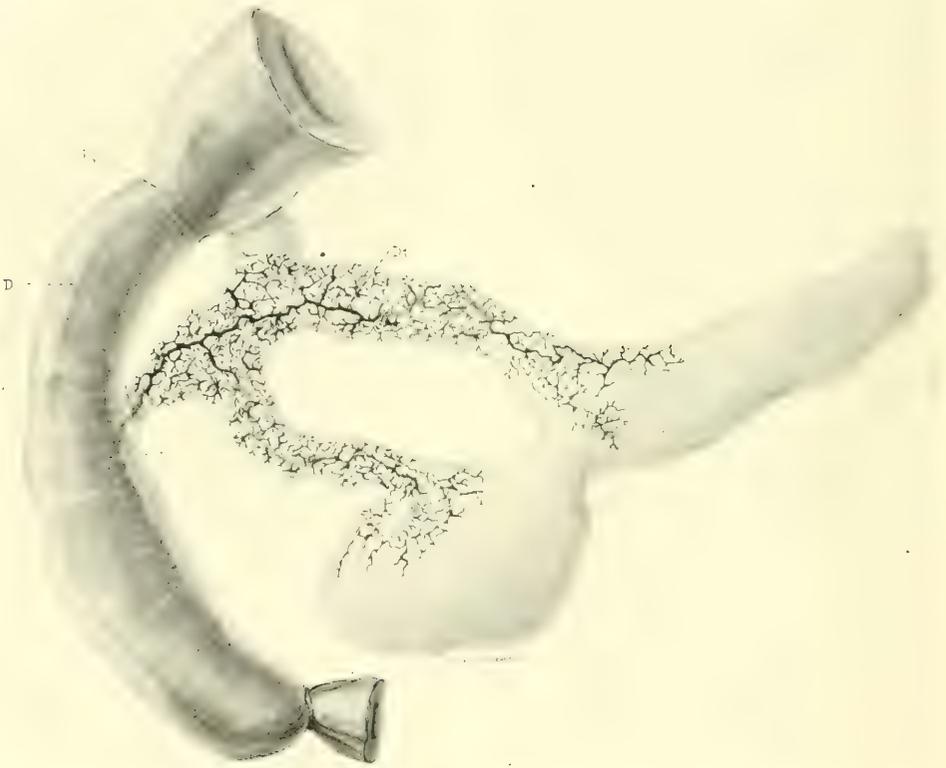


Fig. 9 Pancreas and duodenum of a pig foetus 50 mm. long, ventral aspect; ducts of head of pancreas completely injected. The main duct is now well formed, but the capillary ducts are still plexiform. *D*, duodenum; *Py*, pylorus; *Dt*, main duct. $\times 17$.

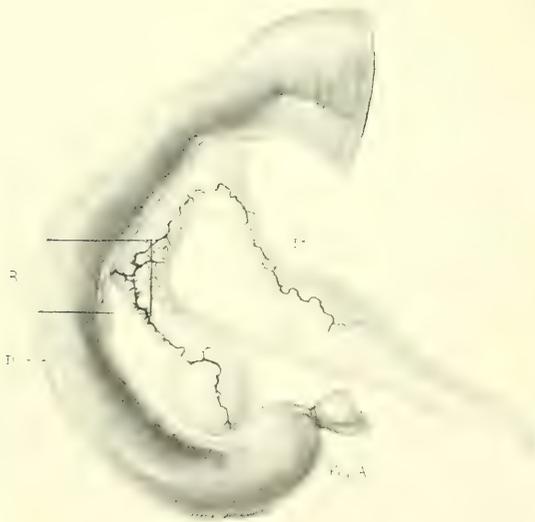


Fig. 10, A Pancreas and duodenum of 60 mm. pig foetus, ventral aspect. Nearly complete injection of ducts, illustrating progress in formation of main duct-channel through the pancreas. $\times 10$.

Fig. 10 B Detail from same as shown by lines. The anastomosing system is beginning to give place to branching, non-anastomosing twigs. *D*, duodenum; *Dt*, panereatic duct. $\times 40$.

fore a flow through the plexus, at a period before the appearance of the main duct.

These observations offer a quite new and very clear explanation of the origin of variations in the pancreatic ducts, which have been a favorite subject of study since the days of Wirsüng and Santorini. The most recent contribution is that of Baldwin ('11) who studied in the human not only the well-known variations as to the presence or absence of an accessory duct, but also

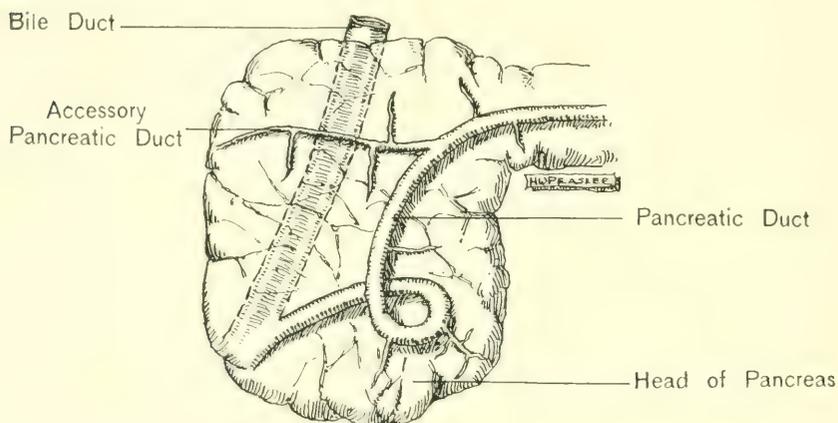


Fig. 11 From Baldwin ('11) showing an abnormal loop found in the main pancreatic duct in three out of one hundred autopsies.

abnormalities in the course of the ducts within the gland. The latter sort of irregularities have always been attributed to abnormal development or growth of one or both anlagen; but to produce some of the anomalies mentioned by Baldwin (fig. 11) the anlagen must have twisted themselves, as they grew, into loops and spirals; yet in these specimens the outer form and relations of the pancreas are normal. The theory falls when we see, as is clearly shown in figure 7, that before the chief duct is formed, the two anlagen are completely fused, so that the anlagen play no great part in the production of variations in the course of the ducts, except so far as they limit the plexus to one, or at most two, points of outlet into the duodenum. On the other hand the primitive plexus explains all the variations, and the abnormalities



Fig. B

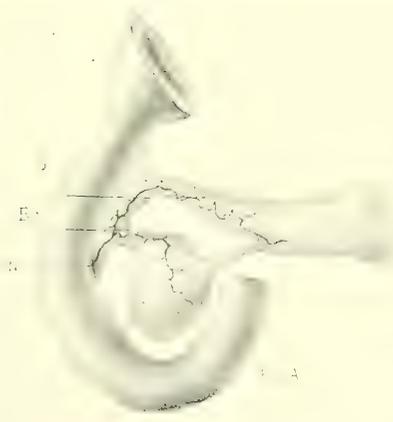


Fig. 12 A Pancreas and duodenum of a 80 mm. pig foetus, ventral aspect. Nearly complete injection of duct-system, excepting tail, made by inserting a fine needle into pancreatic duct at point marked *N* (see p. 221). $\times 4$.

Fig. 12 B Enlarged detail from same as shown by lines. The terminal ducts have branched into complex trees and the anastomoses have disappeared. *D*, duodenum; *N*, point of injection. $\times 40$.

are undoubtedly produced in a way exactly like those of the blood-vascular system, as demonstrated by the work of Evans ('08, '09) and others. Just as a blood-capillary supplying the embryonic arm-bud or kidney may for some reason persist after its time and form an accessory brachial artery or renal vein; so at the time of appearance of the pancreatic duct, varying physical forces may lead the new channel through one or another mesh of the plexus shown in figure 7, and in this way any imaginable variation may be produced, even loops and spirals, while the external form of the pancreas remains without change.

In the 60 mm. stage we find signs of the next step in development (fig. 10). From the sides of the anastomosing tubules grow out many short, narrow twigs, which do not anastomose, and occasionally branch at their tips. In foetuses of 80 mm. (fig. 12), these branching tubules have grown into complex trees, forming the whole organ. Figure 13 *A*, represents a fortunate injection at the 140 mm. stage, made as described, by inserting a fine hollow glass needle into the duct just outside the duodenum at the point indicated (*n*). The India ink has completely filled the ducts, which extend in a branching system all through the organ. In the detailed drawing, figure 13 *B*, the terminal branches are shown in their full complexity, and by comparing this stage with figure 7, one gets a striking impression of the total change which the ducts have undergone.

The former thick plexus has disappeared, and only a few anastomoses remain; they can be found as late as 110 mm., after which they either disappear entirely or are obscured by the forest of acini and ductules. Perhaps they remain in part to form that curious network found by Bensley ('11) about the larger ducts and throughout the pancreas, if his observations upon the guinea-pig hold good for other animals, as we may suppose they do. Why a rapidly developing organ should suddenly quit one manner of growth and take another, is altogether obscure. Perhaps the anastomosing plexus represents a vestigial tendency of the pancreatic anlagen, correlated with their close embryological relation to the liver, to be discarded in accordance with the demands of new function. It is obviously unnecessary for tu-

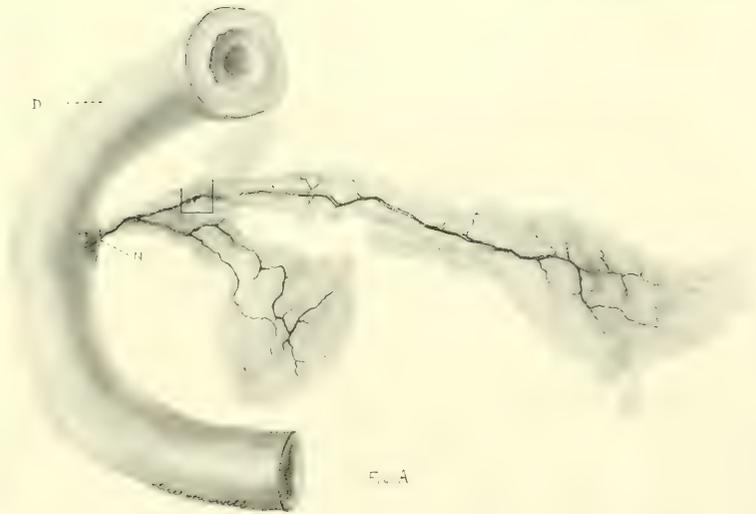


Fig. 13 A Pancreas and duodenum of 140 mm. pig foetus, ventral view; complete injection. The larger ducts only have been shown. $\times 4$.

Fig. 13 B Fully detailed drawing of a portion of the same specimen, as indicated by the lines. The ducts are now entirely tree-like. *D*, duodenum; *N*, point of injection. $\times 40$.

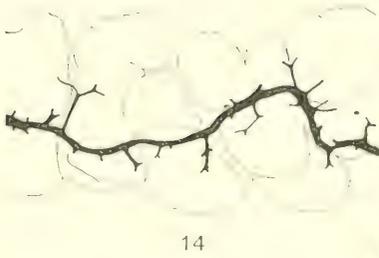
bules like those of the pancreas to have openings at both ends when the flow is entirely in one direction.

Laguesse first found these non-anastomosing buds appearing upon the primitive plexus in sheep embryos, and called them primitive pancreatic tubules. At first they terminate in little branching vesicles. Renault ('99) maintains that there is always one island of Langerhans in each of these clusters of terminal vesicles, and that the whole structure represents a primitive lobule, to become in time a fully-developed lobule of the pancreas, the island being, as it were, its center of growth; to which he adds that the islands become rudimentary in the adult, since their foetal function is lost. These ideas of Renault regarding the islands have not stood the test; but it is true that the lobules arise by growth of the early tree-like outgrowths.

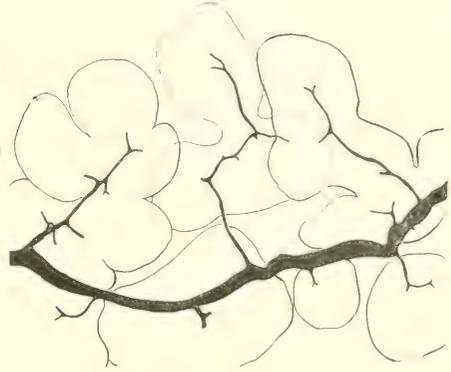
To return to our series: Up to this point the pancreatic tissue has been relatively homogeneous externally, that is, not lobulated. But as the tree-like growth supervenes upon the duct, the tissue becomes rounded off and divided into irregular little nodules, each one of which represents a branch of the duct with its twigs. Further progress is best studied by watching some definite part of the pancreas, for instance, the 'upper bridge' connecting the head and tail of the organ (fig. 1, *UB*). In this spot, in the 80 mm. foetus (fig. 14) the duct is found giving off branches at varying intervals to little rounded masses of tissue of irregular size, which are fused together at many points. In the 110 mm. foetus (fig. 15) there is a little more semblance of order; the masses of tissue are larger, more discrete, and show, in short, a transition to the formation of units as described in the first part of this paper, which is fairly complete in the 'bridge' at 140 mm. (fig. 16).

The increase in the number of units during development (actually counted by teasing, in the foetal stages, estimated in the adult) is roughly as follows:

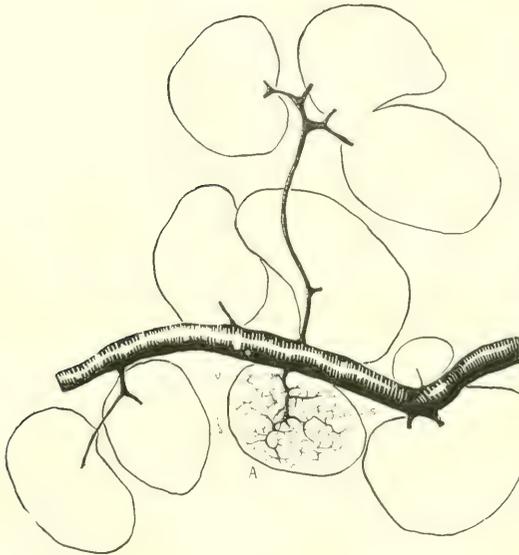
<i>Stage</i>	<i>Number of units</i>
115 mm. length.....	250
140 mm. length.....	400
160 mm. length.....	700
Adult	20,000 to 30,000



14



15



16

Fig. 14 Diagram of 'upper bridge' of 80 mm. pig foetus (fig. 1) showing beginning of formation of structural units as irregularly rounded masses; (*camera lucida*). $\times 35$.

Fig. 15 Diagram of 'upper bridge' of 110 mm. pig foetus, showing progress in the formation of structural units; i.e., the masses of tissue are larger and more discrete. $\times 35$.

Fig. 16 Diagram of 'upper bridge' of 140 mm. pig foetus, showing clear differentiation of structural units. At *A* the ducts of one unit have been shown in full; *u*, unit duct; *s*, straight duct. $\times 35$.

The question arises, how do the few units of the embryo grow into the thousands of the adult, and what determines that the unit shall have a definite size? Laguesse has found that in the sheep the proliferation of cells in the growing embryonic pancreas takes place all along the acinar wall, but chiefly at the distal ends of the acini. Therefore, the growth must be most marked at the periphery of the unit, since the greatest number of acini terminate here. As I have said, the units in pig embryos are of all shapes. A small proportion of them are spherical or ovoid, but most are lobulated by depressions of the surface into from two to five parts. The lobulation may be very slight, or it may have gone so far that it is a question whether to call the bit of tissue one unit, or several (fig. 17, *A, B*). Whenever the lobulation is marked, we find a tiny branch of the artery running into each portion of the unit; the increased tissue area has caused a freer circulation, and the vascular system has responded by transforming a capillary into an arteriole. If the growth continues, our slender arteriole will also grow to supply a capillary net as large as the parent unit; the branch duct which ran to that part of the original unit has become a unit duct; and in this way are formed two, three, four, or five units from one. In the tail of the organ, where the growth in length is to be great, the units generally divide at first into two, end to end, so as to run out long chains of units (fig. 18). In this way rapid extension is gained. At the head, however, density of growth is rather to be obtained, and here the multiplication of units is from the first by fours and fives (figs. 19, 1). Since a spherical form gives the largest volume for a given radius, we find the units rounded as long as there is room for them without compression.

It is very plain that the size of the pancreatic unit is dependent upon the vascular system; the unit contains as much tissue as can be nourished by the capillary bed of one arteriole. In the embryo, in the space between neighboring capillary tufts there is less circulation, and hence the connective tissue, which demands less subsistence than the gland-cells, fills in the space between the capillary tufts. In the embryo the units stand clearly apart, but as the pancreas grows in bulk the tissue becomes

more condensed and the units are crowded together. Anas-
tomoses between the capillary tufts become frequent, the connect-
ive tissue septa are thinned out, and the former clear division
into units is blurred—in some species more than others—so that
in section one sees masses of varying area according to the animal.

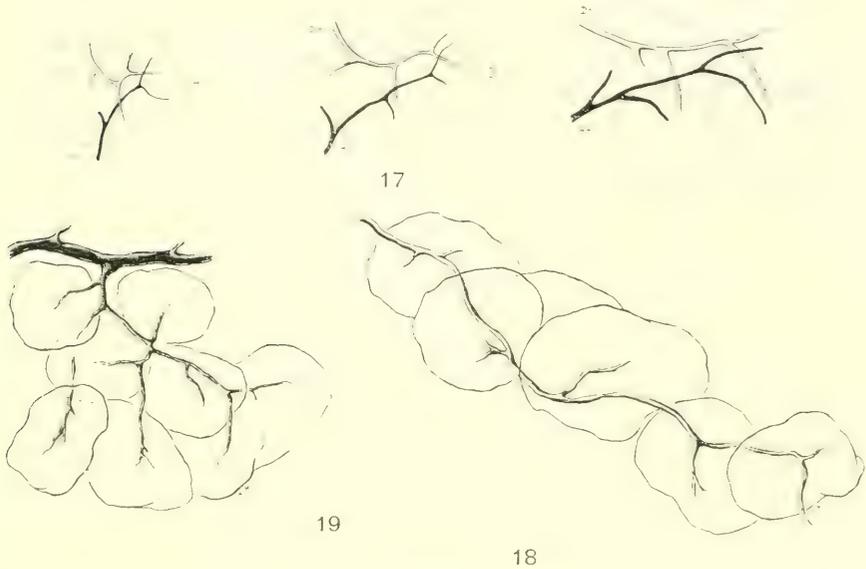


Fig. 17 Diagram to illustrate the growth of one unit into four. *A* is a recon-
struction from actual sections of one unit from a 150 mm. foetus; *B* and *C* repre-
sent the probable growth of *A*. In *A* the duct, *Dt* enters the unit at a point opposite
the artery, *Art*. In *C* the two vessels enter one of the units almost together, *e*.

Fig. 18 Outline drawing of a branch duct and several units from the same
190 mm. pig foetus, illustrating the chain-like method of expansion commonest
in the tail of the pancreas (see also figure 1). $\times 35$.

Fig. 19 Outline drawing of a branch duct and several units from the same
190 mm. pig foetus, illustrating the grape-like method of growth commonest
in the head and body of the pancreas. $\times 35$.

Perhaps a species may be found in which the unit persists clearly
through life, as the unit of the liver in the seal.

Although the blood-circulatory system is the measure which
lays off the duct-tree of the pancreas into regular units, at the
same time, it does not seem unreasonable to suppose that some

of the same laws announced by Thoma to govern the growth of arteries apply also to the growth of the gland-channels. For instance, Mall has given experimental proof of a proposition deducible from Thoma's laws; namely, that all the blood-capillaries of an organ are equally favored by the circulation (Mall '06, p. 249). I have applied the same experiment to the duct system. Having cannulated the pancreatic duct of an adult hog through the duodenal papilla, and having injected a quantity of warm salt solution to flush the ducts, I forced into them a blue solution in small spurts, a little at a time, with low pressure. At first the injection mass did not appear on the surface, but on the fourth spurt the entire organ, with the exception of a few centimeters at the tail, very suddenly became blue, and on the fifth spurt the remainder became blue. Practically all the terminal ducts had filled simultaneously. The conditions of this test are not altogether favorable, since we are reversing the normal direction of flow, but it is clear that all the units are about equally favored with respect to pressure transmitted from the chief duct. The cause of this condition may well be similar to that which brings about the same state in the blood-vascular system; that is to say, the capacity of a duct depends upon the rate of flow of the fluid which normally traverses it. The gland is a sort of hydraulic plant, receiving fluid upon one side from the vessels, delivering it, on the other hand, into the ducts; and as the intake is equal for all the units, so is the output equal.

I have not investigated the nerves and lymphatics of the growing pancreas, as to whether they conform to the unit structure laid off by the blood-vascular system, or not. Studies of these structures offer possibilities of interesting results.

SUMMARY

1. The structural unit is defined as the smallest portion of an organ which is repeated in a similar way throughout, and which contains all the elemental structures of the organ (p. 209).

2. The pancreas of the adult pig is formed by the repetition, 20,000 to 30,000 times, of a structural unit about one millimeter in diameter. The unit is more clearly outlined in the foetus than in the adult. Its size is limited to the area of supply of one arteriole (p. 220).

3. Pressure of fluid injected into the main duct of the pancreas is equally distributed to all the units. By presumption, the reverse is true, that is, all the units deliver their secretion against an equal pressure (p. 234).

4. The pancreatic ducts of the foetus have been injected. Statements of Laguesse and others, that the early pancreatic ducts are plexiform, are confirmed (pp. 221-230).

5. The main duct of the pig's pancreas and its branches arise by dilatation of capillary ducts in the primitive plexus, in a manner similar to the origin of arteries and veins from capillaries. This observation affords a clear explanation of certain variations in the pancreatic ducts, not understood before (p. 226).

I wish to express very hearty thanks to Dr. Mall for his kind encouragement and generous provision of materials necessary to this study.

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AMOEBOID MOVEMENT IN THE CORIAL MELANOPHORES OF RANA¹

DAVENPORT HOOKER

Anatomical Laboratory, Medical Department of Yale University

THREE FIGURES

In the last sixty years, the capability of certain animals to change their coloration at frequent intervals has received considerable attention both from its physiological and morphological aspects. While investigators generally are more or less agreed in regard to various elements which cause such changes, there is still much divergence of opinion concerning the manner in which pigment migration is produced in or by the chromatophore. Many investigators claim that the chromatophores are actively moving amoeboid cells, while an even larger number maintain that they are fixed. Between these two extremes, many intermediate views have been expressed. Indeed, from a review of the literature, it is evident that no less than seven hypotheses have been advanced.

The hypothesis which has had by far the greatest number of supporters, assumes that the chromatophore is a fixed stellate cell within which the pigment, carried in a rather fluid cytoplasm, streams into and out of the processes of the cell during 'expansion' and 'contraction.' This view was first proposed by Brücke ('52) and has since been reiterated by Harless ('54), Virchow ('54), Lister ('58), Solger ('89), Zimmermann ('93 a), Kahn and Lieben ('07), Winkler ('10), Degner ('12), Spaeth ('13) and others.

Ballowitz ('13), working on teleosts, has proposed another hypothesis which is, in a certain sense, a culmination to the foregoing. According to him, the cells are fixed, but the pig-

¹ The writer is indebted to the Francis E. Loomis Research Fund of the Medical Department of Yale University for apparatus and material used in this research.

ment, instead of flowing in a special cytoplasmic medium, occupies a constant intracellular and interanastomosing canal system. Within this closed system the pigment moves out into the processes or back into the cell body. Franz ('08 and '10), on the other hand, would have the pigment granules in teleosts move along constant rods in the cell and thus assume a radial position to the nucleus.

According to Keeble and Gamble ('05), the chromatophores of Crangon are fixed stellate cells having their cytoplasm differentiated "into a firmer, more refractive ectoplasmic wall and a viscous endoplasm." During the process of expansion, "the tubular branches become injected with protoplasm and pigment," while in the contracted phase, "the tubes show up empty and refractive, with here and there a nucleus pressed against their walls."² Such a cell is represented in figure 6 of their plate 1 and shows the pigment-containing cytoplasm contracted into the cell body, leaving an empty space in the processes.

Biedermann ('92), and Ballowitz in his earlier papers ('93 a, b and c), take a middle position. Both these authors claimed that, while the chromatophores may, to some extent, shorten their processes, the latter are never completely withdrawn.

A large number of investigators hold that the chromatophores are amoeboid. The majority of them, however, consider that these cells expand and contract in the intercellular spaces, without any definite path being prescribed for them. Ficalbi ('96) and a few others, express the view that there is no preformed pathway, but that the chromatophores may send out pseudopodia in any direction. This same idea is probably held by many others, such as von Wittich ('54), Busch ('56), Leydig ('57, '73), Hering and Hoyer ('69), G. Pouchet ('76), Ehrmann ('92), Halpern ('91), Fischel ('96) and Verworn ('09).

A. Fröhlich ('10) in a paper on Palaemon, was the first to note that the chromatophores were amoeboid cells which occupied fixed spaces in the tissues, within which they expanded and contracted. He states, "die mikroskopische Untersuchung

² Loc. cit., page 4.

ergibt, dass dort, wo in der Expansionsphase die Ausläufer der spinnenzellenartigen Chromatophoren sich befunden hatten, nunmehr ein Kanalsystem erscheint" ('10, pp. 433-434). It is strange that the fixed space, within which the amoeboid cell expands and contracts, should have been interpreted for such a long time as a fixed cell.

In connection with some work on the color changes of adult *Rana fusca* in the absence of nervous control (Hooker '12), it was noted that the melanophores of this animal occupy preformed spaces. At that time it was not possible to follow up this observation as fully as it deserved. The present paper gives the results of further investigation into the mechanics of pigment movement in both larval and adult frogs.

Rana fusca and *R. pipiens* furnished the adult, and tadpoles of *Rana pipiens* the larval, material used. Over a hundred series of observations were made on the living corial melanophores of tadpoles and adult frogs and preserved material from both was examined microscopically. Meve's solution and corrosive-sublimate-acetic mixture were used as fixing agents, the preparations being later stained in Ehrlich's hematoxylin and congo red, iron hemotoxylin or gold chloride, or were examined unstained.

THE MELANOPHORES OF TADPOLES

The melanophores, which will later come to lie in the corium, first make their appearance in the larvae of *Rana pipiens*, which are 10 to 12 mm. long. They are situated in the subepidermal connective tissue throughout the body and are particularly numerous in the head and back of the tadpole. Those in the head are especially well suited for investigation, as the living tadpole is transparent in this region of the body and as the sheet of connective tissue in which these cells are found may easily be dissected out for microscopic study. The melanophores of frog larvae may readily be examined at high magnifications as they lie nearly in one plane, even when expanded, and parallel to the surface of the body. They will be referred to as the connective

tissue melanophores, inasmuch as they are not strictly corial in the tadpole. Exposure to light causes the melanophores of frog larvae to expand, while confinement in darkness causes them to contract. Both processes are completed in a little less than an hour, so that the changes in form of the cell may be observed in detail and fully controlled.

In the living tadpole, the connective tissue melanophores appear as small, round dots when fully contracted. It is impossible, even with high magnification, to see the cytoplasm and only under the most favorable conditions is a faintly marked, rounded nucleus to be seen lying to one side of the pigment mass. If such cells be exposed to bright light, either from above or from below, they begin to expand. In the living specimen, expansion and contraction of the cell is indicated by the movement of the pigment granules alone, as the cytoplasm is invisible. The pigment begins to stream away in every direction from the previously rounded mass, at first as a fine line of granules, then as a sheet of pigment which increases in breadth until the fully expanded condition is reached.

During the process of expansion of the melanophore, the pigment granules do not move out from the central mass along radiating lines nor do they follow any definite pathway, but move about, now forward, now sideways, now backward, in exactly the same manner as small particles of lampblack suspended in a drop of water which is slowly rolling down an inclined plane. Further, it is noticeable that, in successive expansions of a single cell, its processes are never exactly alike, owing to the fact that it does not always expand at exactly the same rate on all sides.

The completely expanded melanophores of the tadpole are thin, light brown sheets of pigment with short, broad processes of unequal length. A little to one side of the center of the cell is a small area somewhat lighter in color than the rest, although the pigment granules are apparently as thickly distributed here as elsewhere. This is the highly refractive nucleus lying in the cell body. No such pigmentless area as the 'Attraktionssphäre' described by Ballowitz, Solger and others was observed.

In the fully expanded living melanophore, the pigment granules are never at rest, but move about constantly. This fact, together with the absence of any definite channel for their expansion, indicates that the intracellular canal system described by Ballowitz ('13) for the teleosts does not exist in the tadpole.

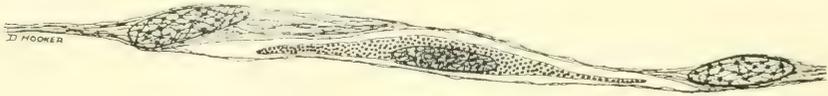
Inasmuch as the tadpoles must be kept in total darkness to obtain contraction of the melanophores, this process may not be observed as readily as expansion. By examining them at intervals, however, it is evident that contraction is merely a reversal of expansion.

The arrangement of the pigment granules in the expanded phase of the larval cell may be seen to advantage in carefully fixed material. They have no definite relation to one another or to the nucleus, but are uniformly distributed throughout the cytoplasm. The nucleus appears here as a dark, rounded area in contradistinction to its light, refractive appearance in the living cell.

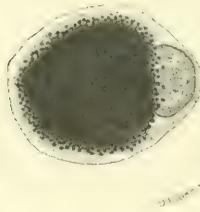
In cross-sections through expanded melanophores, they appear as thin, elongated masses, tapering gradually toward the two extremities and contain, near the center and toward the deep surface of the cell, the nucleus. The latter is in every respect similar to the nuclei of the surrounding connective tissue cells. The pigment granules, when seen in these sections, again do not lie in radiating lines from the center of the cell to the periphery, but are indiscriminately scattered through the protoplasm, which is visible in the preserved material. Such sections also throw light on the relation of these melanophores to their environment. They are situated in an extremely thin sheet of connective tissue cells and fibers which form a series of compartments or spaces (fig. 1) in which the melanophores lie. The walls of these spaces are extremely thin and the cavities themselves have no definite form. Frequently, the cavities overlap at their borders. They are merely connective tissue spaces and, as the melanophores never completely fill them, do not determine the pattern of the expanded cell.

Fully contracted larval melanophores present the following appearance when seen from above (fig. 2): the pigment is massed

in the center of the cell so thickly that its granular nature is visible only at the periphery; to one side of the pigment lies the cell nucleus, usually rounded, though in some cases it appears almost hour-glass shaped; surrounding pigment and nucleus is a thin layer of cytoplasm, faintly granular in appearance which has a definite external border and which presents no sign of processes. Around the outer edge of the cytoplasm and usually separated from it by a clear space is a circle which enlarges or



1



2

Fig. 1 An expanded melanophore from a tadpole of *Rana pipiens* in its space in the connective tissue. Cross-section.

Fig. 2 A contracted melanophore in the subepidermal connective tissue sheet, seen from above. The figure shows the pigment, nucleus and cytoplasm of the cell and the cavity within which it lies.

diminishes in circumference on changing the focus and which represents a section of the convex upper wall of the cavity. Every cell which is fully contracted or nearly so demonstrates this cavity wall. The space contained inside of this line is in no way commensurate in size with the area taken up by the expanded cell.

The reason for this is made clear by cross sections of a connective tissue sheet containing contracted melanophores. Such

preparations demonstrate the cell as a somewhat oval mass of pigment surrounded by a thin layer of cytoplasm and occupying a cavity which is apparently much smaller than that in which the expanded cells lie. Careful observation, however, brings out the fact that the cavity has no less volume than before, but that, with the contraction of the melanophore and the accompanying concentration of its cytoplasm, the superficial and deep walls of the cavity have collapsed peripherally, while in the center, which is now the only part occupied by the cell, these walls have been forced further apart. This has produced, in place of the flat-surfaced cavity, one with convex superficial and deep walls. Seen from the surface, the focal plane of the microscope cuts the rounded upper wall of the cavity and gives the impression of a fairly well-defined ring.

MELANOPHORES OF THE ADULT

In the adult frog, the corial melanophores are found in the skin just below the fixed yellow pigment cells, the xantholeucophores, which, in turn, lie directly beneath the epidermis. In the expanded phase, the processes of the melanophores lie nearly at right angles to the cell body, surrounding the xantholeucophores. This fact, together with the thickness of the skin, renders minute examination of the living melanophore very difficult. The web of the foot was chosen as the most favorable place to examine these cells in the living frog.

In an attempt to overcome some of the difficulties experienced in the study of the melanophores in the live frog, pieces of skin and portions of the web were mounted in plasma according to Harrison's method.³ While some additional details could be thus observed the results were not as satisfactory as had been hoped for, though, for other purposes, such preparations are excellent.

The study of living adult melanophores, both *in vivo* and *in vitro*, demonstrates that each cell has its own pattern which it assumes whenever fully expanded. This fact has been noted by

³ A description of this method and its modifications will be found in the following papers: R. G. Harrison, 1910, *Jour. Exp. Zoöl.*, vol. 9, p. 791; M. T. Burrows, 1911, *Jour. Exp. Zoöl.*, vol. 10, pp. 66-69.

many observers for various animals and has been most recently confirmed by Spaeth ('13) for the teleosts.

When fully contracted, the living melanophore of the adult appears as a rounded mass of pigment, no cytoplasm being visible. Expansion begins in much the same manner as in the melanophores of the larvae. At first, thin streams of pigment granules leave the mass on all sides, gradually growing larger as they extend. Many such processes fuse together and some are withdrawn. It is noticeable that, contrary to the condition in the larvae, these pseudopodia do not coalesce to form a broad



Fig. 3 Two corial melanophores from the skin of an adult *Rana fusca*. In *A*, one, and in *B*, two of the branches of the cavity within which the cell lies are shown. *A* is completely contracted, *B* only partially. In the latter, the cytoplasmic processes at each end of the mass are seen.

sheet, but form elongated, rounded processes. As expansion proceeds, the cell body continually decreases in size and when the extreme is reached, is scarcely to be distinguished from the processes themselves. During the latter part of expansion, the processes lose their uncertainty of direction and always follow the same course to form the individual cell pattern. An expanding cell may, however, begin to contract at any stage, complete or not, in which case the processes are withdrawn again into the cell body.

When fully expanded, the cell appears as a basket made up of a large number of elongated, narrow, rounded processes, some

of which anastomose. Within the 'basket' lie one or more xantholeucophores. The color changes produced are so well known as to need no further description.

The exact path of movement of the individual pigment granules during expansion and contraction is difficult to determine, owing to the thickness of the frog's web or the pieces of tissue in the plasma cultures. No evidence has been obtained, however, that tends to demonstrate the presence of any other type of movement than that observed in the corresponding cells of tadpoles. On the contrary, the two types of movement are very similar.

In cross sections through well fixed frog skin, the fine histological details stand out with remarkable clearness.⁴ One point of difference between the larval and adult melanophores is very evident. Whereas, in the former, the cytoplasm of the cell is almost always visible in fixed material, in the latter, the amount of pigment is so much greater that the cytoplasm may be seen only in those cells which are almost, but not quite, completely contracted. In both the fully expanded and the completely contracted phases the pigment granules fill the cytoplasm so completely as to obscure it. As in the larval melanophores, the pigment granules themselves are not arranged in any definite order, so far as could be determined.

The completely contracted melanophore of the adult appears as a rounded or oval mass of pigment and, like the larval, occupies a fixed cavity. The shapes of the two types of cavities are, however, entirely different. The cavity occupied by the adult cell consists of a central reservoir but slightly larger than the contracted cell, from which tubular branches, one for each process of the expanded cell, extend into the surrounding tissues (fig. 3). As noted in a previous paper, these cavities seem to have an endothelial lining. The lumen of the branches remains patent for varying distances from the cell body, but the tips are usually collapsed, either by intercellular pressure alone, or, as seems

⁴ A Zeiss apochromatic 2 mm. N. A. 1.40, immersion objective and 3, 4 and 12 compensating oculars were used in examining the histological details described in this paper.

more probable, by the withdrawal of the processes toward the center of the cavity as well. The volume contained by the cavity is always the same. The cell bodies of the expanded cells are much smaller than those of melanophores in the contracted phase and the central cavity appears to have diminished in volume, while the processes of the cell have again opened the lumina of the branches.

It is from those cells which are not quite completely contracted that the proof that the cavity is not the fixed cell itself is derived. Such cells (fig. 3, *B*) show the still uncontracted processes, which stand out clearly from the cavity and in which may be seen the cytoplasm of the cell with a few scattered pigment granules in it. It is, therefore, impossible to confuse the cell with its containing space.

In the extreme phase of expansion, the branches of the cavity are completely filled with the protoplasmic processes of the cell. In consequence of this fact, the expansion pattern assumed by each cell is determined by the shape and distribution of the cavity and is in no way assumed by the cell independent of the cavity.

DISCUSSION

Those investigators holding the view that the chromatophores are fixed and immovable cells in whose cytoplasm the pigment streams back and forth, do not attempt to give any idea of the mechanics of such a process, merely stating that within the cells there are at least two types of cytoplasm, which are sharply differentiated from one another, the more fluid of which carries the pigment. While the assumption of the presence of two types of cytoplasm seems justified for the larval melanophores, neither type moves independently of the other. The ectoplasm and the endoplasm bear the same relation to one another that they do in amoeba, moving together in the changes in cell form. If the melanophore, or to make it more general, the chromatophore, were such a cell as is assumed by many investigators, it would certainly be unique in the animal kingdom. The control

by which such a process, alternately causing expansion and contraction of the one type of protoplasm within the other, might go on indefinitely, is by no means clear.

The views advanced for the movement of pigment in teleosts by Franz ('08 and '10) and Ballowitz ('13) are, however, of a much more definite character. Both of these necessitate that the pigment granules should show some arrangement which is constant for each cell. Whatever the condition found in the teleosts may be, neither of these views can possibly hold for the frog. Not only is there no definite movement or arrangement of the pigment granules within the cell, but an exactly opposite condition is present. The clear pigmentless area at the center of the cell mentioned and figured by Solger ('89), Zimmermann ('93 b), and Ballowitz ('93 a) from the chromatophores of teleosts is not visible in the melanophores of the frog under normal conditions. An area lighter, in the living, and darker, in the fixed cells of tadpoles, than its surroundings is very evident, but this appearance is not due to the absence of pigment. On the contrary, pigment granules are present in large numbers. Wilson ('06) speaks of the work of these three investigators as proving the presence of an aster within the cell. From evidence obtained in this investigation, however, it appears that there is no relation between the pigment granules and any such structure, as claimed by Solger and Zimmermann.

The hypothesis of Keeble and Gamble ('05) appears to result from their having overlooked the possibility of the presence of preformed spaces within which the cells may move. This is true for the majority of those who claim that the chromatophore is fixed. They have confused the space with the cell which it contains. The argument offered in favor of the fixed cell idea by Kahn and Lieben ('07), Degner ('12) and Spaeth ('13) is far from conclusive. These authors drew or photographed particular chromatophores in two successive complete expansion phases. That in each case the pictures obtained from the two expanded phases are identical is no proof of the permanency of the processes, for if the cells lie in preformed spaces, the branches

of which they fill in complete expansion, their expansion patterns must always be identical for each given cell. These patterns are forced upon the cell by the cavities in which they move.

In an earlier paper (Hooker '12), the spaces occupied by the melanophores in the frog were termed 'lymph spaces.' This designation was used on account of the close resemblance of these cavities to those in which the corneal cells lie, as described by von Recklinghausen ('62). As a matter of fact, no proof that these spaces contain lymph has been obtained, nor is it of importance in this connection to prove it. However, it seems probable that the spaces contain some fluid, though not in any great quantity.

Ficalbi ('96) believed the chromatophores to be fully amoeboid and to possess the capability of sending out pseudopodia in any direction. The melanophores of frog larvae are just such cells. Their cavities do not limit their motion and all their changes of form are produced by protoplasmic pseudopodia. In the corresponding cells of adult frogs, on the other hand, this capability is hampered by the peculiarities of the containing space. Within the central 'reservoir,' however, the pseudopodia have free play. As any cell which changes its form by means of pseudopodia is amoeboid, the melanophores of the frog are of this type.

Melanophores which have developed in plasma are amoeboid and, not being limited by any space, move about in the medium. This has been previously observed by Harrison ('10). Owing to the fact that many types of cells become amoeboid in plasma cultures, the value of this evidence in the present problem is somewhat doubtful. There is, however, abundant proof of the amoeboid nature of the melanophores from other sources.

RESULTS

1. The pigment granules contained within the melanophores of larval and adult frogs are carried in the cell cytoplasm and not in intracellular canals, along rod-like structures nor in a specialized type of protoplasm. Further they show no definite relation or arrangement to one another nor to the nucleus.

2. The melanophores of both larval and adult frogs lie in preformed spaces in the connective tissue and corium, respectively. The melanophores of adult frogs fill the branches of their preformed spaces in the fully expanded phase, those of tadpoles do not.

3. The melanophores of adult frogs have expansion-phase patterns which are constant for each cell and which are forced upon the cells by their preformed spaces.

4. The melanophores of both larval and adult frogs expand and contract within the spaces which enclose them. As the processes of expansion and contraction are performed by means of pseudopodia, these cells are amoeboid.

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ON THE WEIGHT OF THE THYMUS GLAND OF THE
ALBINO RAT (*MUS NORVEGICUS ALBINUS*)
ACCORDING TO AGE

SHINKISHI HATAI

The Wistar Institute of Anatomy and Biology

ONE CHART

In my recent paper "On the weight of the abdominal and thoracic viscera, ductless glands and eyeballs of the albino rat (*Mus norvegicus albinus*) according to body weight" (Hatai '13), it was not possible to make any statement concerning the thymus gland because its weight is correlated with age rather than with body weight, and the data from rats of known ages were inadequate.

During the last few months, however, these data for the thymus have been collected and the following study is now presented as supplementary to the one cited above.

The investigation has been based mainly on the data obtained by Dr. Jackson from a colony of rats kept at the University of Missouri (Jackson '13). Jackson's records, however, were limited to seven age periods only: birth, 7 days, 20 days, 6 weeks, 10 weeks, 5 months and 1 year. Thus in order to determine the period of the maximum weight of the thymus, as well as to fill the various intervals not filled by him, I have made numerous observations on the Albinos from the colony kept at The Wistar Institute. The Wistar rats, however, were not used until I had tested with data obtained from them the weight of the thymus at the age periods already given by Jackson and found that the two sets of determinations agreed satisfactorily.

I take this opportunity to acknowledge my indebtedness to Professor Jackson, who has granted me the free use of his entire collection of data on the weight of the thymus gland.

The total number of rats used for this investigation was 229 males (165 Jackson and 64 Wistar) and 207 females (179 Jackson

and 28 Wistar). The animals were of the usual size and weight and were in good condition.

As to the method of dissection of the thymus, it is important to remember only that the large lymphatic glands, as well as the fat, which lie close to it, are not to be included in its weight.

The observed weights of the thymus gland for various ages, as obtained from this material, are given in table 1.

Using the observed weights, as given in table 1, formulas have been devised by which the change in the weight according to age may be expressed. Formula 1 applies to rats less than 95 days of age and is as follows:

$$\text{Thymus} = 0.01 \times 10^{1.1} \left\{ 1.1884 + 0.5665 \left(\frac{\text{age}}{55} - 1 \right) - 0.5651 \left(\frac{\text{age}}{55} - 1 \right)^2 \right\} \quad (1)$$

Formula 2 applies to rats 95 days or more of age and is as follows:

$$\text{Thymus} = 0.3903 - 0.00139 (\text{age}) + 0.00000128 (\text{age})^2 \quad (2)$$

Using these formulas we obtain the computed weights of the thymus according to age as given in table 2, and from these the graph shown in chart 1 has been constructed.

As will be seen from the chart, the thymus gland increases in weight very rapidly from birth up to about 85 days in age at which time the gland reaches its maximum. This rapid increase in weight is followed in turn by a steady decrease for the rest of life—the involutionary process. The period of the maximum weight corresponds nearly with the end of the rapid growth of the sex glands in weight, as will be seen from the following relations:

The body weight of the ordinary albino male rat at 85 days of age is 144 grams (Donaldson '06). The corresponding weight of the testes would be about 1.76 grams (Hatai '13). If we take a body weight of 400 grams as the approximate maximum for a fully grown male rat under usual laboratory conditions, the corresponding weight of the testes would be 3.06 grams. From these data we find that nearly 57 per cent of the final weight has been attained by the testes when the rat is 85 days old.

TABLE 1

Showing the weight of the thymus in the albino rat according to age

MALES				FEMALES			
Age	Body weight	Thymus	No. of cases	Age	Body weight	Thymus	No. of cases
<i>days</i>	<i>grams</i>	<i>grams</i>		<i>days</i>	<i>grams</i>	<i>grams</i>	
1	5.1	0.0077	44	1	4.8	0.0076	43
7	10.6	0.0243	30	7	10.5	0.0273	27
20	22.2	0.0845	24	20	17.0	0.0574	25
42	52.9	0.1027	22	42	54.9	0.1134	20
58	137.0	0.3093	6	58	110.7	0.2842	6
72	138.0	0.2287	26	70	103.3	0.2321	23
82	197.0	0.2880	7	85	142.2	0.2830	7
113	218.6	0.2247	12	127	192.0	0.2215	1
122	236.0	0.3083	6	150	142.1	0.2162	21
145	285.8	0.1839	3	159	206.7	0.2130	2
150	167.5	0.2340	20	162	189.5	0.1882	4
159	260.4	0.2473	2	202	194.1	0.1633	4
161	241.8	0.1534	6	215	183.9	0.1696	2
192	281.3	0.1596	4	340	155.1	0.0752	2
209	226.1	0.1223	7	365	163.7	0.0750	20
222	251.6	0.1606	5				
365	213.0	0.0420	5				
			229				207

TABLE 2

Showing the computed weight of the thymus gland with respect to age according to the formulas (1) and (2). Both sexes combined

AGE	THYMUS	AGE	THYMUS	AGE	THYMUS
<i>days</i>	<i>grams</i>	<i>days</i>	<i>grams</i>	<i>days</i>	<i>grams</i>
5	0.0169	80	0.2895	155	0.2056
15	0.0335	83	0.2903	175	0.1862
25	0.0606	85	0.2895	200	0.1635
35	0.0997	87	0.2876	250	0.1228
45	0.1491	90	0.2827	300	0.0885
55	0.2028	93	0.2756	350	0.0606
65	0.2512	95	0.2698	400	0.0391
75	0.2829	105	0.2585	455	0.0228
77	0.2861	125	0.2365	500	0.0153

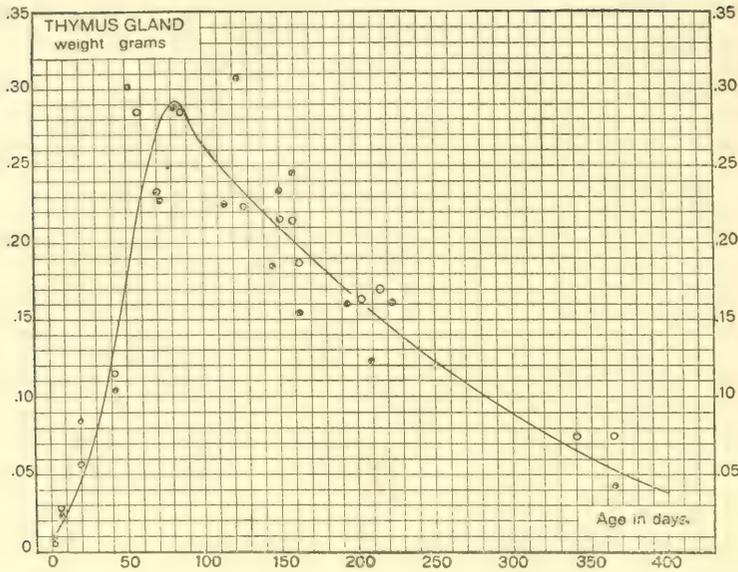


Chart 1 Showing the weight of the thymus of the albino rat according to age. The observed weights are represented by 229 males (165 Jackson and 64 Wistar) and 207 females (179 Jackson and 28 Wistar).

● Male } observed weight | ——— computed weight
○ Female }

Similarly, we find the body weight of the ordinary female rat at 85 days of age to be about 132 grams (Donaldson '06). The corresponding weight of the ovaries is 0.045 grams (Hatai '13). The body weight of 350 grams may be taken as that of the fully grown female rat, and we obtain 0.052 grams as the corresponding weight of the ovaries. From these relations we find that nearly 87 per cent of the final weight has been attained by the ovaries when the rat is 85 days old.

The relations indicated above are of interest in connection with the close physiological association between the sex glands and the thymus, as pointed out by a number of investigators (for literature see Biedl '13; Paton '13; and Vincent '12).

From the chart the considerable variability in the weight of the thymus is clearly seen, a variability the significance of which is increased when we consider the large number of cases which

have been taken, so that in using the formulas it is important to keep this fact in mind. There are several factors which may contribute to irregularities in the weight of the thymus gland. The most important of these, on account of the frequent occurrence under usual laboratory conditions, are the following:

1. Pregnancy and nursing, which tend to decrease the weight of the thymus gland prematurely. Whether or not the rat recovers from this alteration has not yet been determined.

2. Lung infection, and poor growth of body, which are usually associated with a small thymus.

3. During the period of rapid growth (first 85 days) a heavier rat has a relatively larger thymus than a smaller rat of the same age.

Thus though the weight of the thymus is assumed to be a function of age, and the foregoing formulas have been determined on such an assumption, the proper comparison of the observed weight of the thymus with that calculated by the formulas may require several corrections according to the condition of the rat under investigation. For instance, if the observed weight of the thymus according to the formula appears to be too large or too small, the normality of the observed body weight with respect to the age should be determined.¹

If the given body weight deviates noticeably from the normal, the normal age corresponding to the observed body weight should be found. Using the age thus determined, the corresponding thymus weight may be substituted for that directly observed. There may occur still other instances in which the observed weight of the thymus can not be directly compared with the calculated value without making some adjustment necessitated by special conditions. The above instance is given as an example merely.

As was noted before, the thymus gland possesses two distinct peculiarities by which it is contrasted with the other organs; (1) the weight of the thymus is correlated with the age of the

¹ For such purposes see Donaldson ('06) "A comparison of the white rat with man in respect to the growth of the entire body." Boas Anniversary Volume, New York.

rat rather than with its body weight and (2) the thymus gland shows an involutionary change manifested by the diminution in its absolute weight after about 85 days in age. These two peculiarities are both subject to considerable fluctuation which may contribute to the high variability of the thymus in weight.

In order to give some idea of the degree of variability of this organ as contrasted with others, table 3 is presented.

TABLE 3

Showing the coefficient of variation in weight in some organs of the albino rat (compiled from Jackson, '13)

	NEW-BORN	6 WEEKS	5 MONTHS
Total body weight.....	12	21	19
Heart.....	18	30	21
Liver.....	22	19	25
Suprarenals.....	24	22	39
Thymus.....	31	50	22

This table brings out two important facts: (1) The high variability of the thymus, and (2) the fact that the variability is greatest at the period of rapid growth of the body and becomes less as the growth of the body is slowed. We may attribute the greater variability during the first six weeks to the greater variability of the body weight, since as has been mentioned, the weight of the thymus at this period varies not only with age, but also with the body weight, while in the older rats such is not the case. Finally, in the adult rat we can easily eliminate most of the disturbing factors by careful inspection, inasmuch as these are associated in the main with evident pathological conditions.

On account of its great variability, nothing can be stated definitely at this moment as to the sex difference in the weight of the thymus gland. So far as our present data are concerned, the thymus gland of the female appears to be slightly heavier than that of the male, nevertheless, the difference found is too slight to justify treating the sexes separately, so that the same formulas may be allowed to serve for both.

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THE DEVELOPMENT OF THE MAMMALIAN JUGULAR LYMPHSAC, OF THE TRIBUTARY PRIMITIVE ULNAR LYMPHATIC AND OF THE THORACIC DUCTS FROM THE VIEW POINT OF RECENT INVESTIGATIONS OF VERTEBRATE LYMPHATIC ONTOGENY, TOGETHER WITH A CONSIDERATION OF THE GENETIC RELATIONS OF LYMPHATIC AND HAEMAL VASCULAR CHANNELS IN THE EMBRYOS OF AMNIOTES

GEO. S. HUNTINGTON

Columbia University, New York

TWENTY FIGURES

During more than a decade the attention of many morphologists has been attracted to the development and structure of the vertebrate vascular system, and to the interdependent correlations of its individual components.

Our knowledge of the most important genetic phases of the blood-vascular system has been far advanced by the careful and thorough work of many independent investigators. To mention the researches of Weidenreich, Maximow, Mollier, Dantschakoff and Rückert is merely to cite the leaders in a long line of workers who have cooperated in establishing the basic evidence for a genetic interpretation of the haemal vascular system. A careful analysis of the very extensive haematological work of the last ten years, with full consideration of the older investigations, is given by my colleague H. von W. Schulte in a contribution published as No. 3 of the *Memoirs of The Wistar Institute of Anatomy in Philadelphia*. One of the most important subdivisions of the whole field of vascular inquiry deals with the development of the vertebrate lymphatic system and with the relations of the same to the haemal components of the entire vascular apparatus. In its main lines the anatomy of the adult

lymphatic system is fairly well known in the Mammalia. Very important contributions to this subject have recently been made by McClure and Silvester (1, 2). Our knowledge of the adult anatomy of the lymphatic system in the remaining amniote classes is less complete. On the other hand, the development of the lymphatic vessels, in spite of the great progress made in the last ten years, still presents unsettled questions, chiefly because opinions differ as to the genetic relations between lymphatic and blood vascular channels.

The primary questions, which form the basis for the existing differences of opinion, involve the origin of the lymphatic apparatus in the broad sense. Is the same an *independent vascular development*, coequal to and largely concurrent with the development of the blood-vascular system, or is it a *secondary derivative* from the *venous* division of the haemal system? These are the two fundamental questions. There are, of course, many secondary problems which arise at nearly every point in the course of the inquiry into the lymphatic ontogeny of the vertebrates, such as differences in the types of development encountered in embryos of different vertebrate classes, differences in the grade of development, number, structure and location of the lymphatico-venous hearts and of the equivalent lymphatico-venous connections, and many other problems of minor genetic significance. But in the last analysis the question of the independent origin of the lymphatic system, or of its secondary derivation from the veins, remains the cardinal and crucial point upon which the true genetic interpretation of lymphatic organization must be based. We have now, I believe, arrived, on the hand of new and cumulative evidence, at a point where a careful revision of some of our earlier results will materially aid in clarifying the main problem. A brief review of the principal divergent views held regarding the development of the vertebrate lymphatic system may serve to accentuate the significance of the facts I desire to present at this time.

I. The results obtained by the injection of embryos have led some observers to advance—and still maintain—a theory of lymphatic development based on the following postulates:

1. The vertebrate lymphatic system is a direct derivative of the embryonal venous system.

2. At one or more definite points along the course of the embryonal veins lymphatic sacs or 'buds' arise from which, by a process of continuous 'sprouting' of the 'lymphatic endothelium,' the lymphatic vessels proper develop and successively invade the embryonal body in a *centrifugal* direction. From the tips of these lymphatic channels a set of blindly ending closed 'sprouts' are derived which represent the lymphatic capillaries.

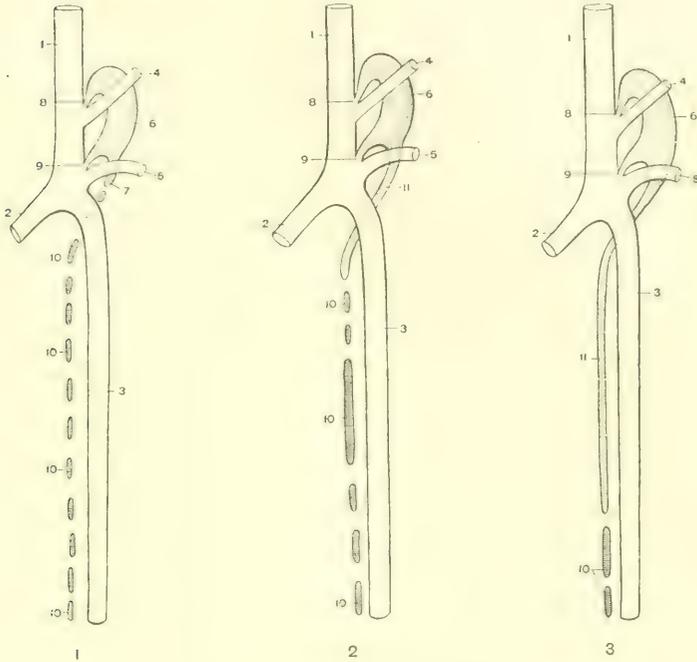
This view involves the acceptance of the theory of 'specificity' of the embryonal vascular endothelium, so that the endothelium lining the early venous channels becomes endowed, by virtue of its 'specific' character, with the faculty of creating, by a continuous centrifugally directed process of sprouting and extension, a system of lymphatic channels which progressively invade the tissues of the embryo from the center toward the periphery. This view may, for the sake of brevity, be defined as the "centrifugal theory of lymphatic development."

I became convinced early in the course of my investigations that Ranvier's view of a *venous* origin of the lymphatic system, as modified by the advocates of the 'centrifugal theory,' was not supported by actual and valid evidence. At that time I pointed out the futility of attempting the solution of the lymphatic problem by injecting already connected and continuous portions of the embryonal lymphatic channel system. The value of the objections then raised against the sweeping conclusions drawn from the partial and incomplete evidence furnished by injections has since then been abundantly and clearly demonstrated.

Injections of the embryonal lymphatics yield easily procured and very beautiful topographical preparations illustrating the *extent* to which, in the individual stages, the independently developed lymphatic anlagen have united into an *injectible system of connected channels*. The method does not in any way touch the *genesis of the lymphatic anlagen* from which these channels arose. Injections, moreover, do not afford the slightest clue as to the presence or absence of mesenchymal intercellular tissue

spaces situated beyond the furthest point reached by the successful complete injection. Such spaces, if they exist and if they are added progressively by accretion to the already connected segment of the developing lymphatic channels, will in the succeeding stages give to the injected area the *appearance* of a continuous centrifugal extension toward the periphery. This appearance is, however, absolutely deceptive and the opposite of the actual genetic process involved in the further growth of the systemic lymphatic channels. McClure's (4) and Kampmeier's (3) reexamination of one of the 'completely' injected pig-embryos of the Johns Hopkins University collection (Series 23A) in serial sections demonstrates unequivocally that beyond the utmost limit of a 'complete' injection of the patent proximal portion of the thoracic duct, independent endothelial-lined spaces exist in the path of the advancing duct, which in the course of further development would have formed increments to its extension, and would, in a slightly more advanced stage, have been included in the injectible area.

While these self-evident facts hardly seem to demand further explanation or illustration, yet my own personal experience has convinced me of the difficulty of conveying the details of lymphatic development to readers only moderately or insufficiently familiar with the appearance of developing lymphatic areas. The important point is to narrow the inquiry to a small number of ascertainable facts. If these facts can be definitely established then correct logical deductions can be based on them. Figure 1 illustrates diagrammatically a stage (14 mm.) in embryos of the cat in which the jugular lymphsac (6) has been formed with its double lymphatico-venous connection, at the common jugular (8) and jugulo-subclavian venous angles (9), and its thoracic duct approach (7). An injection of this portion of the future lymphatic apparatus would correspond to the area shown in stipple in figure 1. The independent lymphatic anlagen (cross-shaded in fig. 1) lying caudal to the thoracic duct approach, in the line to be occupied by the future duct (10), would not be revealed by the injection, although their presence can be determined in serial sections of the injected embryo.



Figures 1, 2, 3.

- 1, Precardinal vein
- 2, Duct of Cuvier
- 3, Postcardinal vein
- 4, External jugular-cephalic vein
- 5, Subclavian vein
- 6, Jugular lymphsac

- 7, Thoracic duct approach of jugular lymphsac
- 8, Common jugular lymphatico-venous tap
- 9, Jugulo-subclavian lymphatico-venous tap
- 10, Thoracic duct anlagen
- 11, Thoracic duct

In a succeeding (15 mm.) stage (fig. 2) a number of the proximal mesenchymal tissue spaces have united with each other and have joined the thoracic duct approach of the jugular lymph sac (11). In the same way longer channel segments have formed further caudad by fusion of a number of the distal anlagen (10). Injection, of the jugular sac in this stage, would show, in comparison with figure 1, a caudal extension of the thoracic duct (11) beyond the postcardinal junction. As revealed by the injection alone the extension might be interpreted, as has been done, as a centrifugal growth or sprout caudad from the jugular lymph sac

to form the proximal portion of the thoracic duct. But examination of the uninjected and uninjectible field caudal to the thoracic duct approach in figure 1 shows that the apparent centrifugal downward growth of the thoracic duct in figure 2 is due to a process of accretion in the reverse centripetal direction of the previously separate and independent tissue spaces (10) into a connected channel which has joined the thoracic duct approach and accounts for the apparent caudal prolongation of the same. In figure 3 (16 mm.) the further caudal extension of the thoracic duct (11), as indicated by the injection, is again in the same way not due to a continuous downward growth, but to the successive accretions to its length obtained by fusion with the distal independent mesenchymal spaces of the previous stages (10).

Taken by themselves and judged solely by the results of the injection test, these three embryos might be regarded as affording proof of the progressive sprouting or growth of a lymphatic channel from the veins toward the periphery. Judged by the examination of the non-injectible area, beyond the limits of complete injection, this entire theory collapses, if it can be proved that in this non-injectible area the earlier lymphatic anlagen exist, which, by successive fusion with each other and with the central portions of the system, produce the lymphatic progression demonstrated in successive stages by the injection. The proof of the existence of these true lymphatic anlagen, constant in occurrence, uniform in location and relations to surrounding structures, and as easily and definitely diagnosed by an experienced eye as the more striking haemal vascular channels, has, I think, been abundantly and conclusively afforded in the last few years by a number of observations (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15).

These investigations cover not only observations on mammalian embryos but also researches on the development of the lymphatic system in the birds and reptiles.

II. The second main theory of lymphatic development regards the lymphatic vessels of the adult vertebrate as the result of successive fusions of embryonal intercellular mesenchymal spaces,

uniting with each other in a *centripetal* direction, until they finally establish their central connection with the permanent venous system. This may be briefly defined as the 'centripetal theory.' In this view vascular endothelium does not possess the character of 'specificity.' Vascular endothelium, wherever encountered, haemal or lymphatic, is the expression of the environmental adaption of an originally isodiametric mesenchymal cell to mechanical influences. Unilateral pressure, e.g., the accumulation of fluid under tension in intercellular spaces, will produce flattened modified mesenchymal cells, which on the one hand confine the fluid, and on the other line, as a continuous layer of endothelium, the tissue space in which the fluid is contained. If the pressure is released, the fluid drained and the intercellular space allowed to collapse, 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, in this view, the modified mesenchymal endothelial cell loses all pretensions to 'specificity.' It cannot extend from the region of its inception toward the periphery unless the mechanical factors responsible for its origin precede or accompany such extension.

This second conception of the basic principles underlying lymphatic genesis in vertebrates has in its broad general applications impressed me most strongly with its truth from the beginning of my investigations, and I have upheld the same on several occasions and in a number of publications. During the last three years further and more detailed study of developing lymphatics in mammals, and extension of the investigation on part of myself and my associates to include the reptilian and avian embryos, have consistently confirmed me in my earlier convictions. At the same time I have realized that certain special phases of lymphatic development deserve the most careful and minute consideration, because the opportunities for errors in interpretation are here most abundant, and because they present many puzzling and, at first glance, contradictory conditions.

I have in mind such problems as the genesis, significance, number and distribution of the adult lymphatico-venous connections or 'taps,' including the consideration of the lymphatico-venous hearts and their modified and reduced representatives. Further, the tentative valuation of the *physiological* factors which in the successive stages have an important bearing on the determination of the *morphological* type of the developing vascular system as a whole, and of the lymphatic component of the same in particular. Another important question involves the comparative study of the apparently divergent and dissimilar conditions which, under the same general genetic law and following the same ground plan, are observed in embryos of the three amniote classes. This inquiry includes an attempt to correctly define the mutual structural and physiological relations existing genetically between the haemal and lymphatic components of the general vascular system in embryos of the Amniote vertebrates.

To the supporters of the first of the general theories of lymphatic development (the 'centrifugal theory') above briefly outlined (pp. 260-264) the question of the formation, significance, number and distribution of the adult lymphatico-venous connections in any given vertebrate becomes a matter of relatively secondary importance as regards the genesis of these connections.

If the vertebrate lymphatic system develops as the result of a process of continuous centrifugally directed outgrowth, or budding, or sprouting from the preëxisting endothelium lining the embryonal veins, and if this process can start from one or two, or four, or a larger number of points along the embryonal venous pathways, then it becomes evident that in the establishment of the adult lymphatico-venous connections the development must have followed one of two lines: One or more of the original points from which a lymphatic system of this type sprouted from the venous system either (a) retained the original connection of the sprout or sprouts with the veins, as the future avenue or avenues of the adult lymphatico-venous connections, or (b) separated temporarily from the parent endothelial venous channel, and then reëntered the same secondarily to establish

the adult lymphatico-venous connection or connections. Both of these views seem to have been entertained, at different times, by the advocates of the centrifugal theory.

If, on the other hand, the vertebrate lymphatic system develops according to the tenets of the second or 'centripetal' theory above outlined, the question of the establishment of the adult lymphatico-venous connections becomes exceedingly important. If independently developed para-haemal lymphatic anlagen develop as intercellular spaces in the embryonal mesenchyme and then unite with each other to form a connected and continuous system of lymphatic channels, this system must make necessarily secondary connection or connections with the veins at definite and constant angles of venous confluence, corresponding to the known adult types of lymphatico-venous communication. In the embryos of all three amniote classes this secondary connection is effected in the anterior part of the body through the jugular lymph sac, representing a modified and rudimentary anterior lymph-heart.

The development of this structure has been studied in the mammalian embryo by Sabin (16, 17) (pig, man), F. T. Lewis (18) (rabbit) and McClure and myself (19) (cat). My colleague, Prof. A. M. Miller, has traced its developmental history in the chick (20), and I have done so in chelonian and lacertilian embryos (12).

All of these investigations revealed a very close and intimate association of this segment of the lymphatic apparatus with the pre- and postcardinal veins adjacent to and including their confluence at the Cuvierian junction.

The early anlagen of the jugular lymph-sacs in mammalian, avian and reptilian embryos appear as an intricate plexus of vascular channels and spaces, for the most part filled with blood cells and in certain stages in free communication with the systemic veins.

When McClure and I published the results of our joint investigation on the development of the jugular lymph-sacs in embryos of the domestic cat, in 1908 and 1910 (19), we described these vascular anlagen of the sacs as occupying a *dorso-lateral* position

in reference to the main pre- and postcardinal venous lines, in sharp contrast to a regularly disposed metameric series of *dorso-medial* somatic tributaries draining into the pre- and postcardinal veins. In the later stages we noted that the *dorso-lateral* elements became confluent to form a large sac, filled with blood and connected with the systemic veins at one or more points. This sac then suddenly evacuated its contents of free blood cells into the veins, separated for a short period from the latter, and then made a secondary connection with them at one or both of two typical points, viz., the angle of confluence of the internal and external jugular veins (common jugular tap) and the jugulo-subclavian angle (subclavian tap).

In the succeeding stages these sacs made connections with the systemic lymphatic channels, the thoracic ducts, and the lymphatic trunks accompanying the internal and external jugular, the innominate, cephalic and deep cervical veins, and the broncho-mediastinal lymphatic channels. We found that these systemic lymphatic vessels formed as the result of successive centripetal fusion of numerous intercellular mesenchymal spaces, lined by a lymphatic endothelium and developed independently chiefly along the course of the principal veins, or, as notably in the case of the anlagen of the thoracic duct and of the mesenteric lymphatics, replacing topographically early embryonal pathways which in course of further development retrograded or disappeared entirely, e.g., the left azygos vein, the extrapericardial portion of left duct of Cuvier, the left post-caval vein.

This genetic history of the mammalian jugular lymph sacs, repeated closely in its main lines in avian and reptilian embryos, impresses on these structures a peculiar and definite character, quite distinct both from the true venous channels and from the independently developed systemic lymphatic vessels. In their early anlagen they appear more closely related to the veins, in their later stages they form an integral part of the amniote lymphatic apparatus. Their evident topographical and morphological correspondence to the completely developed lymphatico-venous hearts of the lower vertebrates suggested that they functioned in this modified and reduced form in the same way, as

links or bond-pieces between the independently formed system of the lymphatic channels proper and the veins, and I have thus interpreted them in several previous publications (8, 9, 21, 22, 23).

At the time when McClure and I published our results in detail (10) the question as to the 'venous' or 'non-venous' origin of the vertebrate lymphatic system was still an active one. The only criterion applied at that period of the investigation to the diagnosis of an embryonal lymphatic as against a venous vessel was the presence within the lumen of the latter of free red blood-cells. As above stated the early anlagen of the jugular lymph sacs become filled with blood-cells and communicate freely with the channels of the systemic veins. We consequently at that time regarded these early anlagen as *direct derivatives* of the veins.

Two facts, however, impressed themselves upon us even then during the progress of our investigation:

1. We were always able to differentiate sharply between the regular series of the metameric *dorso-medial* pre- and postcardinal *somatic* tributaries, forming definite components of the permanent venous system, and the more irregular and plexiform *dorso-lateral* mass of vascular spaces, filled with blood for the most part, which we recognized as the anlagen of the future jugular lymph sacs, and which we consequently defined as the '*veno-lymphatics*.'

2. Among these latter we observed, described and figured in numerous embryos of the earlier stages closed endothelial-lined sacs, some containing red blood-cells, others entirely empty. We interpreted the former as either accidental separations from the general veno-lymphatic plexus, or as elements which had not yet joined the same. The latter we regarded as possibly representing veno-lymphatic elements which had, on a small scale, anticipated the evacuation of the blood contents and the separation from the veins which becomes typical for the entire dorso-lateral veno-lymphatic plexus in the succeeding stages. Up to this point the *direct derivation* of the mammalian *jugular lymph sacs* from the early *pre- and postcardinal veins*, adjacent to and including their Cuvierian junction, has not been questioned.

The more recent investigations in this field, covering a wider range of vertebrate embryos, have, however, yielded such decisive results that we are now enabled and entitled to make a closer, and in my opinion more correct, analysis of the mutual relations between developing haemal and lymphatic channels.

From this new standpoint the significance of the jugular lymph sacs in the general plan of the entire vascular system must be revised and distinctly modified.

It is of course evident that the same corrected and modified interpretation must be assigned to all lymphatico-venous connections of adult vertebrates homologous to the amniote jugular lymph sacs, whether they appear as distinct and fully developed lymphatico-venous hearts or as more or less reduced and modified representatives of these structures.

While these recent investigations of the early developmental stages of the vertebrate vascular system have made it possible to take a more generalized view of the phylogenetic and ontogenetic relations of the lymphatic to the haemal department of the general vascular system, they have, at the same time, thrown much additional and new light on the probable functional significance of the primitive vascular channels, and on certain physiological phases of the early vertebrate lymphatic apparatus. The researches here cited have been in part already published, while the remainder still await publication. I owe it to the courtesy of my colleagues and associates that I have been privileged to follow the progress of their work and am permitted to refer to some of their results at this time. The published papers are as follows:

F. A. STROMSTEN 1910 A contribution to the anatomy and development of the posterior lymph hearts in turtles. Publication no. 132 of the Carnegie Institution of Washington, pp. 77-87.

1911 On the relation between mesenchymal spaces and the development of the posterior lymph hearts in turtles. *Anat. Rec.*, vol. 5, April, pp. 173-178.

1912. On the development of the prevertebral (thoracic) duct in turtles as indicated by a study of injected and uninjected embryos. *Anat. Rec.*, vol. 6, pp. 343-356.

GEO. S. HUNTINGTON 1911 The development of the lymphatic system in reptiles. *Anat. Rec.*, vol. 5, pp. 261-276.

- A. M. MILLER 1913 The development of the jugular lymph sacs in birds. Amer. Jour. Anat., vol. 12, pp. 473-491.
 1913 Histogenesis and morphogenesis of the thoracic duct in the chick; development of bloodcells and their passage to the blood stream via the thoracic duct. Amer. Jour. Anat., vol. 15, pp. 131-163.
 1913 Hemophoric function of the thoracic duct in the chick. Science, N. S., vol. 37, June.
- J. E. McWHORTER and A. O. WHIPPLE 1912 The development of the blastoderm of the chick *in vitro* Anat. Rec., vol. 6, pp. 121-139.
- W. F. ALLEN 1913 Studies on the development of the veno-lymphatics in the tail-region of *Polistotrema* (*Bdellostoma*) *stouti*. First communication: Formation of the caudal hearts. Quart. Journ. of Microsc. Science, N. S., no. 234 (vol. 59, Part 2) July, pp. 309-360.
- S. FEDOROWICZ 1913 Untersuchungen über die Entwicklung der Lymphgefäße bei Anurenlarven. Bullet. de l'Acad. des Sciences de Cracovie, Serie B, Juin, pp. 290-297.

The investigations, some of whose results I am permitted to quote, pending their forthcoming publication, are the following:

- C. F. W. McCLURE: The development of the lymphatic system in fishes. Presented to the Section on Anatomy and Embryology, 17, Intern. Med. Congress, London, August 6 to 12, 1913. (Extract to be published in the Proceedings of the Congress, full paper elsewhere.)
- H. VON W. SCHULTE:¹ Early stages of vasculo-genesis in the cat (*Felis domestica*) with especial reference to the mesenchymal theory of endothelium. Memoirs Wistar Institute of Anatomy, No. 3, 1914.
- A. M. MILLER and JOHN E. McWHORTER:¹ Experiments on the development of blood vessels in the area pellucida and embryonic body of the chick (Anat. Rec., 1914).
- RANDOLPH WEST: The development of the posterior lymph hearts of the chick.
- GEO. S. HUNTINGTON: The development of the lymphatic channels draining the anterior limb in embryos of the domestic cat. Memoirs Wistar Institute of Anat., no. 4, 1914.

The titles of some of these researches are still subject to revision and alteration. Wherever possible I have indicated the probable date and place of forthcoming publication. As far as their direct or corroborative testimony affects the problem here under consideration, I am able to summarize some of the results in the following brief statements:

1. The amniote embryo presents, in certain definite regions and during definite ontogenetic stages, aggregations of mesen-

¹ During the process of publication of this article both of these papers have appeared, the former as No. 3 of the Wistar Memoirs (pp. 1-52), the latter in Anat. Record, vol. 8, no. 4, pp. 203-227.

chymal cells which include all phases of developing blood cells, differentiated *in situ* from the indifferent mesenchymal syncytium.

2. These aggregations of developing blood cells are especially rich in erythrocytes.

3. The blood cells are conveyed into the circulating stream of the permanent blood vessels in one of two ways:

a. Temporary haemal vascular channels form around the blood islands, and after the latter's resolution, carry the free blood cells directly into an adjacent main venous trunk.

This type is seen in mammalian embryos, especially in the axial line, during the existence of the temporary ventro-medial tributary plexus of the azygos veins, which plexus then atrophies and is secondarily replaced by the independently developed perivenous anlagen of the thoracic ducts.

It is also encountered in the ventral mediastinal region where an early perithymic plexus is replaced by the broncho-mediastinal lymphatic trunk, and in the development of the mesenteric lymphatic plexus.

b. The early haemopoetic embryonal mesenchyme develops in regions not directly associated with the permanent veins. The blood cells are then conveyed to the general haemal circulation by primitive lymphatic channels, which, after the performance of this function, either remain as integral components incorporated in the lymphatic apparatus, or retrograde and ultimately disappear.

The early lymphatic system assumes therefor in these embryos a special physiological duty, which we have defined as the *haemophoric function*, in contrast to the *haemopoetic* character of the early mesenchymal syncytial areas furnishing the free blood cells.

A. M. Miller (26) has already defined this early haemophoric function of the axial lymphatic trunks of the chick in the preliminary note above quoted, and his extensive publication appeared in the September issue of *The American Journal of Anatomy* of last year (15).

McC lure, in a detailed study of lymphatic development in the teleosts finds that in the trout lymphatic channels function more extensively and for longer ontogenetic periods as blood-carriers

than is the case in amniote embryos. A résumé of the results of his investigations has appeared in the Proceedings of the Seventeenth International Medical Congress.

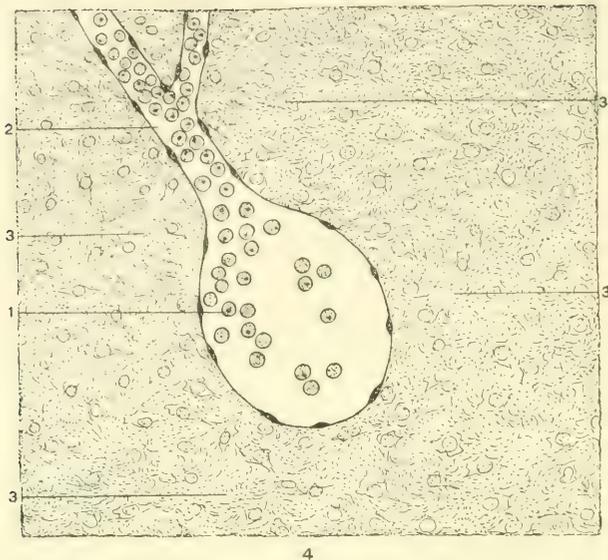
In mammalian and reptilian embryos my own researches of the past three years have convinced me of the existence of precisely the same physiological and morphological conditions in the area of the jugular lymph sacs and some of their tributaries.

I can, perhaps, best illustrate these conditions, with reference to the previous publications on this subject, by the following series of purely schematic diagrams, whose details, however, are amply supported by the study of serial sections and reconstructions. In the very early stages in cat embryos the precardinal veins (1) and their main dorso-medial somatic tributaries (2) appear clearly defined in the uniform mesenchymal field (3) (fig. 4).

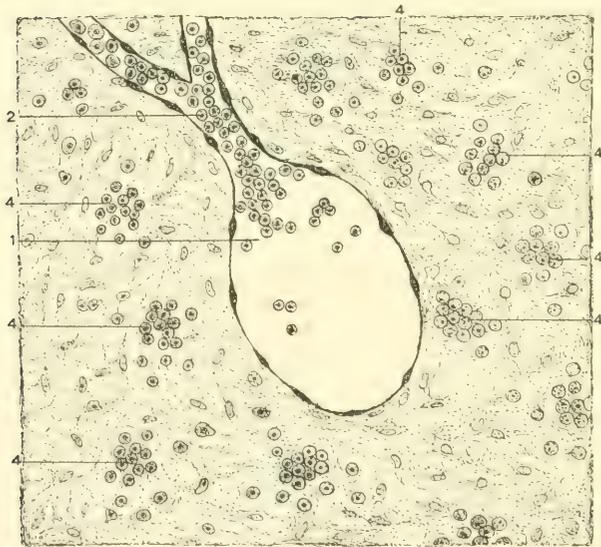
Subsequently (fig. 5) the mesenchymal area dorso-lateral to the main precardinal line shows numerous small collections of developing blood cells (4). These cell-masses are clearly differentiated from the free blood cells circulating in the veins by their characteristic reaction to the Mann stain and by the fact that they gradually fade off on their periphery into the indifferent mesenchyme. They are not surrounded by an endothelial envelope, but appear to be imbedded directly in the mesenchymal syncytium from which they arose. They are as a matter of fact nothing else than intraembryonic representatives of the early vascular strands of the extraembryonic area vasculosa. This stage is of very brief duration and is followed by a transitional or prehaemophoric stage (fig. 6) in which a number of intercellular clefts (5) appear in the mesenchyme, lined by endothelium and in close apposition to the blood-islands.

This phase practically offers a replica of the pictures obtained by Whipple and McWhorter in their study of the developing vascular system in the blastoderm of the chick *in vitro* (27), and by Schulte in the publication on the early stages of vasculogenesis in the cat, with especial reference to the mesenchymal theory of endothelium (Wistar Institute Memoirs, no. 3, 1914).

In the succeeding stage (fig. 7) the isolated intercellular mesenchymal spaces have united into an intricate plexus (8) whose



4



5

Figure 4

Figure 5

- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 3, Perivenous mesenchymal syncytium

- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 4, Mesenchymal blood-islands

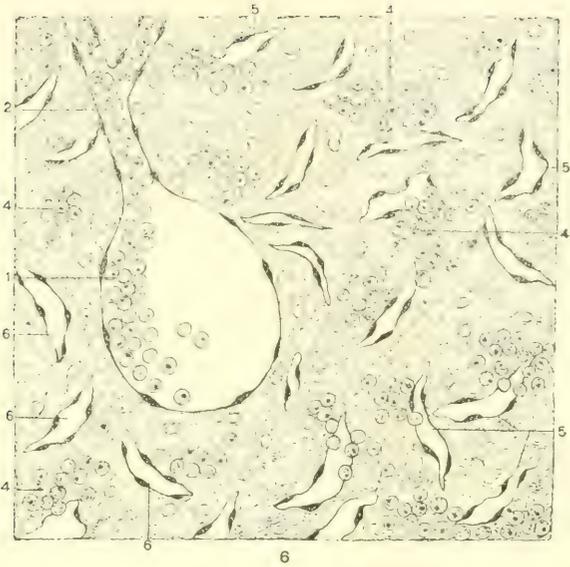


Figure 6

- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 4, Mesenchymal blood-islands
- 5, Dorso-lateral mesenchymal intercellular spaces, forming anlagen of jugular lymphsac
- 6, Medial mesenchymal intercellular spaces, forming anlagen of brachio-cephalic venous anastomosis

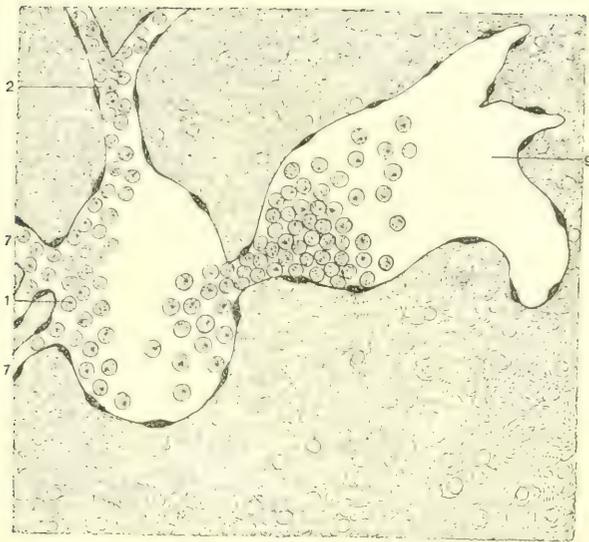


Figure 7

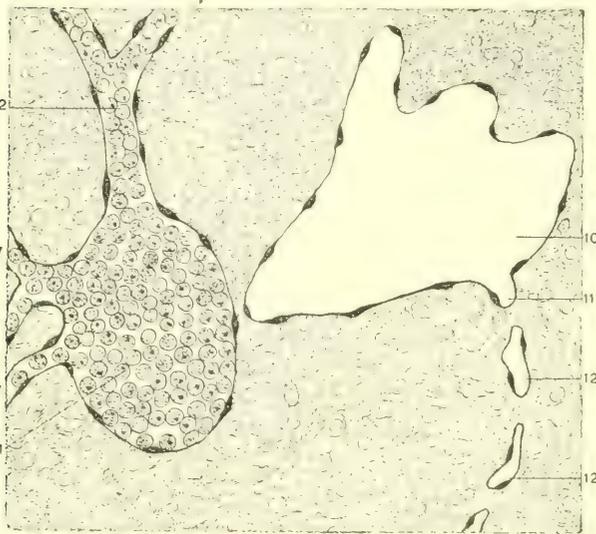
- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 7, Brachio-cephalic venous anastomosis
- 8, Dorso-lateral haemophoric lymphatic plexus, haemophoric recipient stage of jugular lymphsac

walls are lined by a distinct endothelium. The meshes of this plexus have surrounded the earlier blood-islands, the individual cells of the latter now appear free in the lumen of the plexoid channels, and these have established at numerous points (10 to 14 in some of the embryos described and figured by McClure and myself) connections with the pre- and postcardinal veins. This stage, represented schematically in figure 7, I have designated as the *haemophoric recipient* to indicate the fact that independently formed channels have united into a plexus, established connections with the veins and are beginning to collect the blood-cells of the adjacent haemopoetic mesenchyme, for the purpose of conveying them to the venous circulation. This is the first phase in the haemophoric function of the early mammalian lymphatics. In the following period (fig. 8) the spaces and meshes of the dorso-lateral veno-lymphatic plexus have united into a multilocular sac (9) which is engaged in pouring its contents of free red blood-cells into the systemic veins. The period corresponds to what McClure and I described in 1910 as the 'stage of evacuation,' and which I will here define as the '*haemophoric evacuating stage*,' in contrast to the '*haemophoric recipient stage*' of figure 7. The subsequent periods develop the complete evacuation of the jugular lymph sacs (10) and their temporary separation from the venous channels (fig. 9), the development of the independent peri-venous anlagen of the thoracic duct and the systemic lymphatics (12, 14), and the secondary approach of the lymph sac to the main veins at the definite points of the future lymphatico-venous connections (fig. 10).

Lastly (fig. 11) the lymphsac establishes, on the one hand, its definite and now permanent connections (16) with the venous system at either the common jugular or the jugulo-subclavian venous angles, or at both of these points, while on the other it receives the independently developed paravenous systemic lymphatic trunks, viz., the thoracic duct (17), the jugular, cephalic, deep cervical and broncho-mediastinal trunks (18). The sac now serves as the common portal of entry of the connected lymphatic system into the haemal division of the entire vascular complex.



8



9

Figure 8

- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 7, Brachio-cephalic venous anastomosis
- 9, Jugular lymphsac, haemophoric evacuating stage

- 2, Dorso-medial somatic tributary
- 7, Brachio-cephalic venous anastomosis

Figure 9

- 1, Precardinal vein

- 10, Jugular lymphsac, stage of complete evacuation and of temporary detachment from the veins
- 11, Thoracic duct approach of jugular lymphsac
- 12, Thoracic duct anlagen

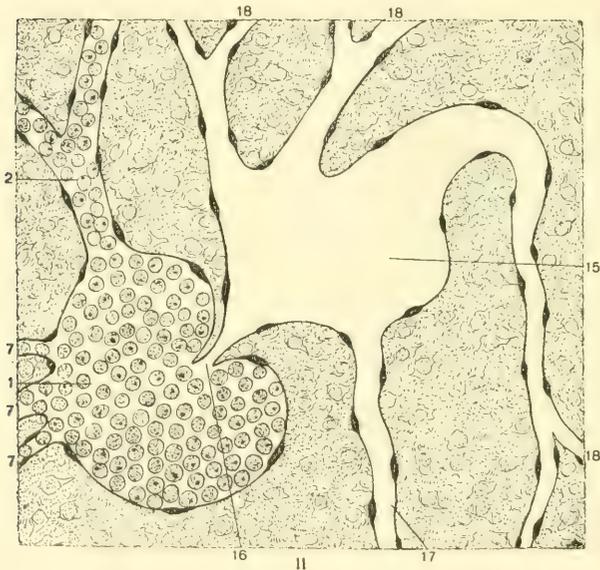
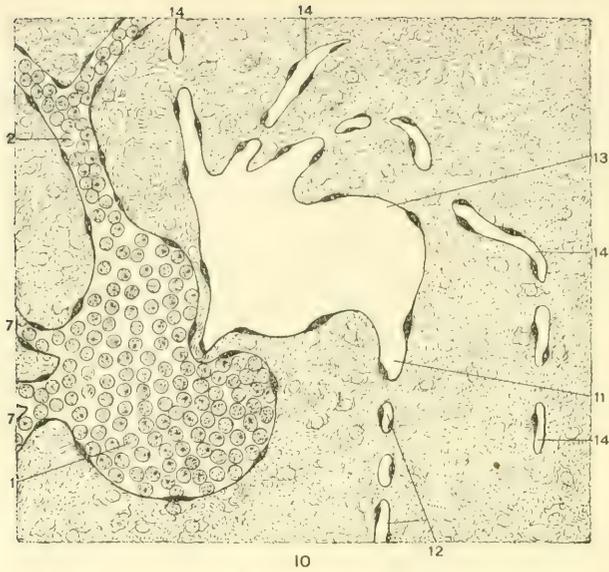


Figure 10

Figure 11

- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 7, Brachio-cephalic venous anastomosis
- 11, Thoracic duct approach
- 12, Thoracic duct anlagen
- 13, Jugular lymphsac, preparing to reenter veins and to establish secondary connections with anlagen of thoracic duct (12) and of other systemic lymphatics (14)

- 1, Precardinal vein
- 2, Dorso-medial somatic tributary
- 7, Brachio cephalic venous anastomosis
- 15, Jugular lymphsac
- 16, Permanent lymphatico-venous tap
- 17, Thoracic duct
- 18, Jugular and cephalic systemic lymphatics

The foregoing offers a very concise and much abbreviated history of the jugular lymphsacs as viewed from the standpoint of the more recent investigations.

McClure and I, publishing our detailed results in 1910, started our investigation of the genesis of the sac about at the stage shown in figure 7 of the above series of schemata.

In spite of the fact that we noted, described and figured the detached and isolated blood-islands and the independent intercellular mesenchymal spaces of the earlier stages, the whole picture, in the absence of the correlated evidence since then obtained by the study of other amniote embryos, and without the recently introduced special blood stains, forced us to adopt the view that the anlagen of the jugular lymph sacs were primarily *venous* derivatives from the pre- and postcardinal veins. We would today define them as *primary lymphatic channels*, developed, as are *all true lymphatic vessels, independently* of the veins, but charged during a definite embryonal phase with the physiological duty of conveying the products of mesenchymal haemopoiesis to the circulating stream of the veins. They thus appear, from their inception, as components of the future lymphatic system, sharing the same type of independent genesis with all the remaining lymphatic channels, but temporarily *haemophoric* in their physiological relation to the vascular system as a whole.

I have in a number of previous publications formulated a genetic interpretation of the development of the mammalian lymphatic system which, in the light of the more recent investigations, should be modified in one respect. Instead of regarding the jugular lymphsacs, the connecting links between the independently developed systemic lymphatics and the veins, as being primarily *venous* in their derivation, it would be, in our present knowledge, more correct to define them as *primitive lymphatic channels* whose early *haemophoric function* serves to differentiate them sharply as a group from the remaining systemic lymphatics, developed at a later period through the same genetic confluence of intercellular mesenchymal spaces, but without direct relation to the veins or to the blood-contents of the haemal system. It is here in all probability that the clue is to

be sought to the somewhat puzzling temporary separation of the jugular lymph sacs from the veins. This separation marks the termination of their primary haemophoric activity. After the process is accomplished their detachment permits them, on the one hand, to join the systemic lymphatics, while on the other it enables the then active rearrangement of the permanent venous system to take place, unimpeded by direct connections with the lymphatic terminals.

A comparison of this developmental history of the jugular lymph sac of the higher vertebrates, representing in these types an *anterior* or *cranial* lymphatico-venous heart, with the results obtained in recent investigations of the development of the *posterior* or *caudal* lymphatico-venous heart, yields exceedingly interesting and corroborative evidence in favor of the genetic interpretation above outlined.

The development of the caudal lymphatico-venous heart has recently been studied by Allen (29) in *Polistotrema* (*Bdellostoma*) *stouti*, by Stromsten (25, 26) in the loggerhead turtle, *Thalassochelys caretta*, and by Fedorowicz (30) in Anure larvae (*Rana esculenta*, *R. temporaria*, *Bufo vulgaris*, *B. viridis*). West, working in the morphological laboratories of Princeton and Columbia Universities, has conducted a reinvestigation along modern lines of the development of the caudal lymphatico-venous heart in the chick.

Allen (29) finds that the caudal lymphatic hearts of *Polistotrema* (*Bdellostoma*) *stouti* develop by the formation of isolated mesenchymal spaces in the region of the anterior ends of the two branches of the caudal vein. These spaces develop by the breaking down of certain mesenchymal cell processes in the centre, and the thickening of others to form the boundaries of the cavity. Subsequently the mesenchymal partition between the spaces and the caudal vein breaks down, establishing a secondary lymphatico-venous connection of the caudal lymph heart with the caudal vein, while the more distal isolated mesenchymal spaces develop, increase in size, unite and join the mesenchymal cavity already connected *secondarily* with the caudal vein.

The above, which is practically a literal transcript from Allen's paper, fully covers the first point in the ontogeny of vertebrate lymphatico-venous hearts, i.e., *the development of the lymphatico-venous heart cavity* in the following stages:

a. Development of isolated mesenchymal spaces, and their fusion to form the heart anlage.

b. Establishment of a secondary connection between the heart anlage and the adjacent caudal vein, by the breaking down of the "mesenchymal partition between the spaces and the caudal vein."

c. Accretion in the centripetal direction of the "more distal isolated mesenchymal spaces" to the "mesenchymal cavity already connected secondarily with the caudal vein."

Allen here offers a very concise and clear picture of the development of a lymphatico-venous heart. There is no 'budding' from the caudal vein, nor any 'sprouting' from the 'venous endothelium.' Heart-cavity and limiting border-cells develop independently of the venous system, by union of the isolated mesenchymal spaces and by modification of the boundary cells (*vide infra*).

Moreover, the heart thus formed establishes a *secondary* connection with the adjacent caudal vein, while *distally*, new "isolated mesenchymal spaces develop, increase in size, unite and join the mesenchymal cavity already connected secondarily with the caudal vein."

This is progressive centripetal accretion of lymphatic anlages as found everywhere in areas in which lymphatic development is active. Allen further expresses himself clearly in reference to the second and third points of morphological importance, viz., the *contents* of the heart cavity above described, and the genesis of the cells *limiting the cavity*: He states that "in places the border mesenchymal cells are flattening to contribute to the endothelium of the heart, while certain of the enclosed cells increase in size, become spherical and differentiate into red corpuscles." Here we encounter, in the first place, the independent development of lymphatic endothelium, lining the early mesenchymal lymphatic spaces. This endothelium has not 'sprouted'

from preëxisting endothelium, it has no connection with any haemal endothelium, venous, arterial or capillary, and it never had such a connection. Let us hope that observations, such as Allen's, if sufficiently and laboriously multiplied, may finally eradicate from 'modern' anatomical and embryological textbooks, such as the recent Keibel-Mall publication, that unfortunate dogma charged to Rabl, "Alles Endothel stammt von Endothel!"

Allen's work further touches on one phase of the early intra-embryonic haemopoiesis. He finds that, within the early lymphatic anlagen, "certain of the enclosed cells increase in size, become spherical and differentiate into red corpuscles." Speaking of the lymphatic or veno-lymphatic organization of *Polistotrema* he states that "in all of the larger or medium-sized embryos the connective tissue outside these (lymphatic) vessels was so filled with red corpuscles as to resemble germination centres, and the vessels themselves appeared to be reservoirs for storing them."

Allen, on page 350 of his paper, sums up the development of the caudal hearts in *Polistotrema* as "a vacuolation of the mesenchyme by the disintegration of certain of the mesenchymal cell processes, and the flattening of some mesenchymal cells to become endothelium, and a rounding of others to become red corpuscles." This description is in complete agreement, in every important morphological aspect, with Miller's discovery of the development of the avian axial lymphatics (15, 27).

Stromsten, in two publications (25, 26) describes the development of the posterior lymph hearts in turtles (loggerhead turtle, *Thalossochelys caretta*). He states (26, p. 175) that:

The development of the posterior lymph hearts is initiated in the loggerhead turtle by the vacuolation of the subcutaneous mesenchymal tissue of the post-iliac regions. As early as the middle of the second week . . . the mesenchyme becomes very loose and spongy. Toward the close of the second week, the mesenchymal spaces enlarge and fuse with each other. There is thus formed a network of intercommunicating spaces which invest and communicate with invading capillaries from the first two or three post-iliac branches of the post-cardinals.

By the middle of the third week of development, the dorsolateral branches of the postcardinal veins have fused at their distal ends to

form the caudal branches of the renal advehent system. The formation of this pair of veins produces profound changes in the post-iliac spongy area. Great spaces or channels appear. These spaces seem to be enormously dilated capillaries which intercommunicate with each other, and connect with the accompanying veins at one or two points. They contain red blood cells, and are indistinguishable from the veins of that region, except by their position. They correspond to the veno-lymphatics of birds and mammals.

It is evident that Stromsten is here describing the formation of early lymphatic anlagen by the fusion of isolated intercellular mesenchymal spaces. The resulting channels then become *haemophoric* or 'veno-lymphatic,' and then establish their connections with the venous system.

Stromsten lays no stress on the early phases of the intra-embryonic haemopoiesis, and his account does not deal with the inclusion of red blood cells developed *in situ* within the lumen of the early lymphatic channels, nor with the latter's haemophoric function in conveying these blood cells to the haemal circulation. Still, his description tallies so closely with the results of Miller's, West's and Allen's investigations that the presumption of identical genetic processes becomes more than probable.

Fedorowicz, (30) in a preliminary communication presented to the Academy of Sciences of Cracow by Hoyer on June 2, 1913, deals with the early development of lymphatics in the region of the caudal lymph hearts in *Anura*. He describes the development of cell strands, on the ectal surface of the lymphatic hearts, in which strands intercellular spaces appear, finally resulting in the formation of an extracardial tubular structure whose lumen is lined by independently developed endothelial cells. By continued extension of the space formation and of the endothelial modification of the limiting cells, a complete lymphatic vessel develops. This vessel has at first no connection with the caudal veno-lymphatic heart. In the lumen of the latter, which Federowicz regards as a dilated evagination of the lateral caudal vein, similar cell strands appear, which gradually acquire a lumen, by the development of intercellular spaces, and become lined by endothelium developed *in situ*. Finally the lumina of the strands developed in the later stages *within* the cavity of the

'heart' unite secondarily with the lumina of the *extracardiac* strands first developed. Federowicz states that the lymphatic vessels in the immediate neighborhood of the hearts cannot be demonstrated by injection prior to the establishment of the above outlined connections. Federowicz's preliminary account is very brief and not completely clear on some points. Part of the difficulty of his presentation seems to lie in his recognition of the fact that his results do not agree with those of previous observers, among whom he quotes Köllicker, Golubew, Rouget and Clark. His evident disinclination to disagree with these investigators, in spite of his divergent results, leads him, however, to make some curious and contradictory statements. He thus attempts to argue from his findings for a *centrifugal* extension of his early independent extracardial lymphatic anlagen. The parental wish seems to influence this thought to a degree which seriously detracts from its scientific value. He states on page 296 of his preliminary communication, above quoted, as follows:

Vergleicht man nun den oben geschilderten Prozess der Zellstrangbildung aus der Wand des von der Venenwand entstandenen Lymphherzens, die Ausbreitung der Stränge in der Umgebung der Herzen, das Entstehen der Lichtung in denselben, die Klappenbildung und die Vereinigung der Lichtung der Stränge mit der des Lymphherzens, mit dem später an derselben Stelle ausgebreiteten Lymphgefässnetz, so kam man nur den Schluss ziehen, dass dasselbe aus jenen Zellsträngen hervorgegangen ist.

So far Fedorowicz describes his own observations in the development of cellular mesenchymal strands, the intercellular formation of spaces within these strands, their union to form lymphatic vessels of longer extent, lined by a lymphatic endothelium developed *in situ*, and the establishment of secondary connections in a *centripetal* direction of these extracardial early lymphatic spaces with the intracardial spaces of the lymphhearts. This primary union of the independent lymphatic anlagen takes place from the pericardial periphery toward the interior of the heart. If a *centrifugal* process were involved it would seem that this is the crucial point for its manifestation and that the early lymphogenesis would proceed from the lymphheart toward the

periphery. Instead, Fedorowicz first establishes the independent ontogeny of the lymphhearts and of the extracardiac early lymphatics. Then these are brought together, their lumina connecting, not directly, but through the intervention of a somewhat complicated system of cellular strands which develop, later than the extracardiac lymphatics, within the cavities of the heart, acquire an intercellular lumen and then secondarily connect with the lumina of the extracardiac lymphatics. All this speaks of a most determined effort on the part of the independently developed extracardiac lymphatics to gain entrance, proceeding centripetally, into the interior of the lymphheart. And yet, immediately following his account of these events, and after mentioning that the early lymphatic plexus often exhibits a definitely radiating arrangement, he proceeds to state: "Es wäre kaum möglich anzunehmen dass diese Gefässe von den beiden longitudinalen Lymphgefässen des Schwanzes, der *V. lymph. longit. dors. et ventralis*, nach dem Herzen zu so regelmässig zentripetal wachsen könnten." It seems difficult to see why, once accepting the idea of 'growth' in a continuous line, the same should not take place in either direction, from the center toward the periphery, or reversely from the periphery toward the center. Nor can I see any reason why such growth should be more 'regelmässig' if directed centrifugally. Fedorowicz assumes two possible points for starting his theoretical growth, one centrally from the 'heart,' the other peripherally from the "dorsal and ventral longitudinal lymphatic trunks," and decides in favor of the former apparently on the ground that it would be impossible to conceive of growth taking place so 'regularly' in the reverse direction. This is pure assumption, and not evidence. Any vertebrate embryo can 'grow' in any of his parts just as 'regularly' from the center toward the periphery as from the periphery toward the center, as far as any biogenetic law is concerned of which I have knowledge. The fact that the embryo 'grows,' or more euphemistically 'develops,' in either of both of these directions, in establishing his main static developmental lines, has nothing to do with the ontogeny of the embryonal anlagen which enable these processes to take place. It seems

inexcusable to Fletcherize an idea to this extent, but I feel compelled to again point out that the difference between the *centrifugal* and the *centripetal* theory of development of the vertebrate lymphatics is not merely one of *direction*. The former involves the concept of a *continuous* uninterrupted centrifugal extension of lymphatic growth from one or more central foci, in this case the endothelium of certain venous channels. The centripetal theory is based on the development of the finally continuous lymphatic channels by the union of separate and independently developed lymphatic anlagen into a connected vascular system, in exactly the same way as the earliest anlagen of the blood vessels unite into a continuous whole. Observation shows that the *direction* of this union in case of the lymphatic system is from the periphery toward the center, but this element is of less morphological importance than the actual process of lymphatic vasculo-genesis.

Apparently Fedorowicz has never considered the possibility that between his extracardial lymphatic anlagen and the longitudinal lymphatic trunks of the tail there may exist a series of independent intercellular lymphatic mesenchymal spaces whose union with each other and with the central and peripheral links of the chain will merely repeat the process of secondary junction which he has so carefully described between the earliest lymphatic extracardial anlagen and the cavity of the lymph heart. At any rate, failing the proof that such intermediate connecting anlagen do not develop, and in view of the fact that the only phase which Fedorowicz has observed and described is the centripetal union of independently developed extracardial lymphatics with the hearts, it is difficult to see the validity of his conclusions:

Ueberdies müssten sie (the extracardial lymphatics) entweder schon vorhanden sein ehe die Herzen angelegt würden (why?) oder sie würden an den Herzen erst sichtbar sein wenn sie an dieselben von der Peripherie herangewachsen wären. (why?) Somit sind die an den Lymphherzen sich bildenden Zellstränge als Anlagen der Lymphgefäße anzusehen, die von den Herzen nach der Peripherie auswachsen.

If these conclusions were true, why do the earliest extracardial lymphatics at first extend from the extracardial area

into the heart? Do they then reverse their direction and grow out toward the periphery? What histogenetic evidence has Fedorowicz in support of the supposition that they thus grow until they unite with the longitudinal dorsal and ventral lymphatics of the tail? Also, where do these vessels come from?

Fedorowicz's work, as above stated, suffers from the attempt to correlate his results to the divergent views of previous investigations. Thus he says (p. 296), in speaking of the earliest lymphatic anlagen as observed by him: "Freilich entwickeln sie sich an den Herzen in anderer Weise, als dies Kölliker, Golubew, Rouget und Clark beobachtet haben." Then he continues: "Doch wurden die *unzweifelhaft richtigen* Beobachtungen dieser Forscher erstens an lebenden Material und ferner an den feinsten Lymphgefäßen ausgeführt."

It is difficult to see where Fedorowicz finds grounds for reconciling his results with those of the investigations he quotes. Two sets of observations cannot possibly contradict each other and still both be right, provided they cover the same premises. If an observation on fixed material differs from one made on living material, then one of three things has happened: Either the observation on the fixed material is correct and the observation on the living material is wrong, or the observation on the fixed material is wrong and the observation on the living is correct, or, lastly, both the observations on the fixed material and the observation on the living material are wrong. Only one fact appears to be absolutely certain, viz., the observation on the fixed material and the observation on the living material cannot possibly both be correct if they furnish fundamentally different conclusions. Still, in spite of this conflict with authority, Fedorowicz finds the earliest anlagen of the lymphatic vessels in cellstrands, which then acquire a lumen by the development of intercellular spaces, and subsequently make secondary connection with the lymph hearts.

These observations, coming from Hoyer's laboratory, which has for so long maintained the centrifugal outgrowth of the lymphatic system from the veins, are encouraging indications of a coming impartial attitude on part of the Polish investigators.

West has studied the development of the caudal lymph heart in embryos of the chick with fully injected blood vessels. His results will shortly be published in full. I am permitted to quote the following conclusions from the summary of his paper:

1. The mesenchyme lateral to the caudal muscle plate, and caudal to the hind limb bud, presents in 10.5 mm. embryos, between the points of penetration of the first five coccygeal veins, an enlargement of the interstitial spaces, which soon fuse with one another, by the breaking down of the mesenchymal processes, to form disconnected uninjectible lacunae, bounded by mesenchymal cells which become flattened to form an endothelium.

2. The lacunae adjacent to the lateral branches of the first five coccygeal veins establish secondary connections with these veins just lateral to the point at which the veins penetrate the caudal muscle plate, while more distally developed mesenchymal spaces successively add themselves to those already connected with the veins.

3. Simultaneously with the formation of the mesenchymal lacunae the adjacent mesenchyme exhibits areas of active haemopoiesis, in which mesenchymal cells in all transitional stages of differentiation into erythrocytes and lymphocytes are found. In addition, some erythrocytes develop directly from the endothelial cells lining the mesenchymal spaces.

4. The red blood cells thus formed become included within the lumen of the lymphatic lacunae in one of two ways:

(a) Some blood cells find their way into the lymphatic lacunae by a constant breaking down of the mesenchymal processes during the enlargement of the lymphatic spaces.

(b) Other blood cells gain direct access to the interior of the lymphatic channels by migration through the latter's endothelial walls.

These intralymphatic red blood cells are conveyed into the haemal circulation through the lateral branches of the first five coccygeal veins.

Thus the lymphatic plexus, which subsequently forms the posterior lymph heart, has assumed at this stage of its development not only a *haemopoetic*, but also a *haemophoric* function.

We have here a group of four independent observers investigating the development of the caudal lymph hearts in a wide range of vertebrate embryos, avian, chelonian, anure amphibian and cyclostome. The results of all four investigators show a remarkable unity of conclusions. They do not all touch on every phase of the problem, but their combined findings yield, for the interpretation of the ontogeny of the caudal lymph hearts of vertebrates, of their connections with the systemic lymphatic

vessels, on the one hand, and of their communication with the veins, on the other hand, the following conclusions:

1. The formation of the caudal lymph hearts is inaugurated by the development of independent intercellular mesenchymal spaces in the immediate neighborhood of main somatic or appendicular venous trunks.

2. The individual intercellular mesenchymal spaces unite to form a cavity of larger size, and the walls of this cavity are lined by a *lymphatic* endothelium, developed *in situ*, by the flattening of the mesenchymal cells along the border of the cavity. This *lymphatic endothelium* is developed absolutely independently of any other preexisting endothelium, haemal or otherwise. It has no connection with the endothelium lining the blood vascular channels, and it never had such a connection. It is not the result of 'budding' or 'sprouting' or 'proliferating' on the part of any embryonal endothelium whatsoever, but it is simply and solely the result of the response which the embryonal mesenchymal cell returns to a mechanical and physical impulse. If two embryonal mesenchymal cells are separated from each other by the accumulation of fluid in the resulting intercellular space, then the opposing aspects of the two cells involved will be subjected to the mechanical and hydrostatic influences of accumulated intercellular fluid, which will react upon the surfaces of the cell still held in syncytial relation to the surrounding mesenchyme. The cells whose opposing surfaces have become freed by the development of an intercellular space, and are subjected to fluid pressure, will react as a whole, become flattened, and be transformed into endothelial cells, forming the parietal limit of an originally intercellular mesenchymal space, which is the font and origin of all vertebrate vascular development, independent of the question as to the ultimate destiny of the space in the organization of the vascular system of which it forms a part. The particular space in question may become a constituent of the heart, of the arterial, of the venous, of the haemal capillary, or of the lymphatic components of the entire vascular complex. This is a matter of individual differentiation in an individual vertebrate embryo. In all four accounts there is *no evidence*

of a centrifugal extension of vascular endothelium by sprouting or otherwise. Fedorowicz's attempt to argue for a centrifugal extension of his early independent lymphatic anlagen has been discussed above.

3. The resulting lymph hearts establish secondary connections with the adjacent veins.

4. The independently formed lymphatic mesenchymal spaces and the resulting plexuses contain red blood cells derived from three sources: (a) Some mesenchymal cells contained in the center of a developing lymphatic cavity enlarge, become spherical and are directly transformed into red blood cells. (b) Other red cells develop by the direct transformation of the border endothelial cells lining the early lymphatic spaces. (c) The mesenchyme surrounding the early mesenchymal spaces is actively haemopoetic. These red cells gain access to the lymphatic lumen either by extension of the space formation to include and surround the resolving blood-islands (West), or by direct migration of the free red cells through the loose wall of the lymphatic endothelium (Allen, West).

5. The lymphatic channels receiving free red blood cells from these sources convey them to the venous circulation. They are in this stage *haemophoric* channels.

The four papers above quoted agree therefore with the lines laid down by Miller (15, 27) for the development of the avian thoracic duct in the early haemopoetic and haemophoric stages. They all present a striking congruence with the above outlined developmental history of the anterior or jugular lymph sac in mammals, birds and reptiles.

Returning to the consideration of the conditions obtaining in the mammalian embryo, the outline of the genetic history of the jugular lymphatic sac previously given (pp. 273-280) may be further supported by the detailed developmental study of one of its main tributary channels, the primitive ulnar lymphatic (figs. 12-17).

With the development of the anterior limb-bud the lateral vein of the body wall receives the blood returning from the extremity through a marginal vessel. The combined trunk then

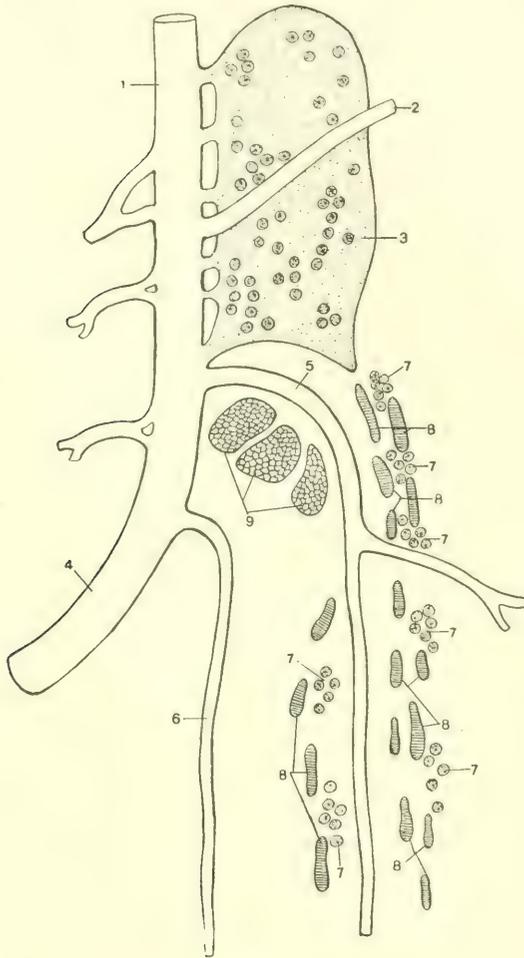


Figure 12

- | | |
|-----------------------------------|---|
| 1, Precardinal vein | 7, Mesenchymal blood-islands |
| 2, External jugular-cephalic vein | 8, Intercellular mesenchymal spaces,
the anlagen of the primitive ulnar
lymphatic |
| 3, Jugular lymphsac | 9, Brachial plexus |
| 4, Duct of Cuvier | |
| 5, Primitive ulnar vein | |
| 6, Postcardinal vein | |

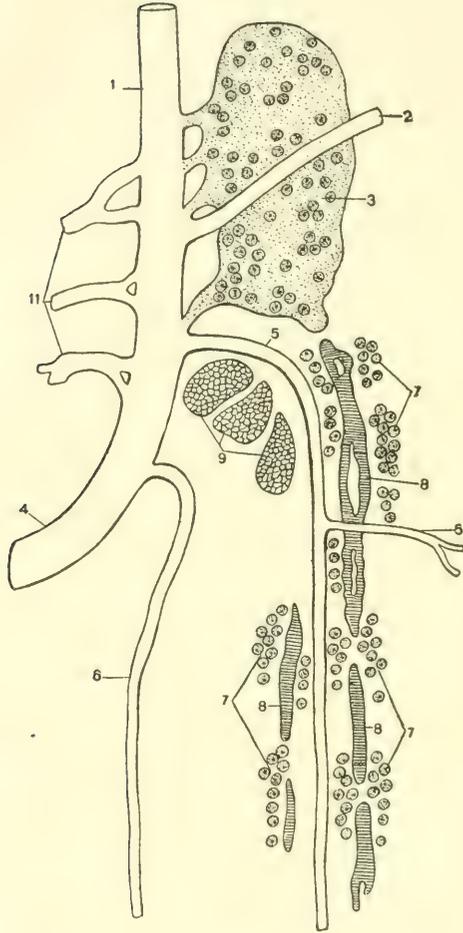


Figure 13

- | | |
|-----------------------------------|---|
| 1, Precardinal vein | 7, Mesenchymal blood-islands |
| 2, External jugular-cephalic vein | 8, Anlages of primitive ulnar lymphatic |
| 3, Jugular lymphsac | 9, Brachial plexus |
| 4, Duct of Cuvier | 11, Brachio-cephalic manastomosis |
| 5, Primitive ulnar vein | |
| 6, Postcardinal vein | |

ascends as the primitive ulnar vein (5), arches over the sixth spinal nerve (9) and enters the dorsal aspect of the precardinal vein (1) (jugular promontory), in close relation to the caudal end of the jugular lymphsac (dorsal division, 3, fig. 12). Along the dorso-medial circumference of this proximal segment of the primitive ulnar vein a series of intercellular clefts develop in the mesenchyme (8). These clefts enlarge rapidly, unite with each other and form a channel, the primitive ulnar lymphatic, which parallels the vein of the same name (8) (fig. 13). At this time for a short period the primitive ulnar lymphatic contains no blood cells, but is distended with clear fluid and lined by a layer of flattened cells which are assuming endothelial character. The blind cephalic end of the lymphatic channel extends to within a short distance of the caudal extremity of the jugular lymphsac, but is as yet not connected with the same. In the mesenchyme surrounding the primitive ulnar lymphatic along its entire course are numerous small collections of blood cells in all stages of development (7, figs. 12 and 13).

The primitive ulnar lymphatic appears in this condition in cat embryos between 8 mm. and 9 mm. and even up to the 9.5 mm. stage. During this period the channel still reveals its formation through fusion of a large number of separate and originally independent collections of intercellular mesenchymal spaces. This accounts for the occurrence at intervals in the course of the main channel of multilocular and plexiform areas. In these areas the character of the cells limiting these primitive spaces ranges from the typical indifferent mesenchymal cell to a complete and distinct endothelium. The mode of development of the primitive ulnar lymphatic also accounts for the reduplication of the channel frequently observed, two parallel canals running side by side for longer or shorter distances.

In the succeeding stage (10 mm. to 10.5 mm.; fig. 14) the primitive ulnar lymphatic (10) becomes filled with red blood cells derived from the clumps of developing blood-islands which lie in the mesenchyme in close propinquity to the lymphatic channel. The lumen of the latter extends to include and surround these areas of developing blood cells. After their reso-

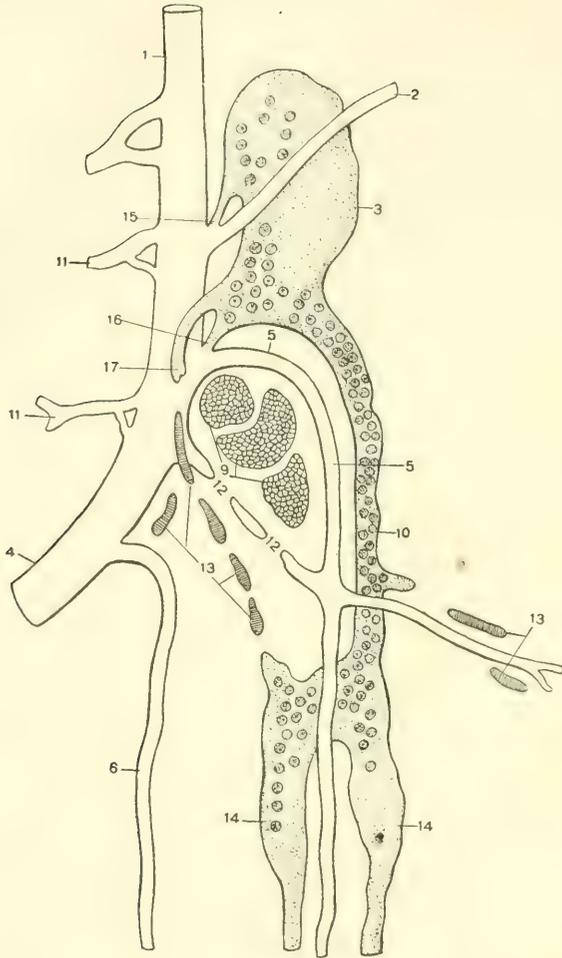


Figure 14

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|---|--|
| 1, Precardinal vein | 14, Anlagen of subclavian or axillary lymphatic sinus |
| 2, External jugular-cephalic vein | 15, Common jugular lymphatico-venous tap |
| 3, Jugular lymph sac | 16, Jugulo-subclavian lymphatico-venous tap |
| 4, Duct of Cuvier | 17, Ventral prolongation from subclavian approach of jugular lymphatic sac to meet subclavian and mediastinal systemic lymphatics (18) |
| 5, Primitive ulnar vein | |
| 6, Postcardinal vein | |
| 9, Brachial plexus | |
| 10, Primitive ulnar lymphatic (haemophoric stage) | |
| 11, Brachio-cephalic anastomosis | |
| 12, Anlagen of subclavian vein | |
| 13, Anlagen of subclavian lymphatics | |

lution the free erythrocytes pack the lymphatic channel densely, so that it often appears greatly distended. In some embryos, caught at the critical and very evanescent stages, portions of the plexiform or reduplicated segments of the lymphatic channel are found filled with blood cells, while adjacent segments are still empty. This again is the *haemophoric recipient stage* in the development of the primitive ulnar lymphatic. As soon as it is attained the distended and blood-filled lymphatic vessel establishes a connection with the caudal end of the dorsal division of the jugular lymph sac (fig. 14, 3). It now corresponds in every respect to the veno-lymphatics of our earlier descriptions, and hence McClure and I defined this structure in our joint papers in 1908 and 1910 as the "primitive ulnar veno-lymphatic," in the absence of any detailed knowledge of its earlier genetic stages.

The channel next pours the blood collected in it rapidly into the large reservoir of the jugular sac, through which it gains access to the systemic venous circulation (fig. 15). This process signals the *haemophoric evacuating stage*. As soon, almost, as the evacuation is accomplished (embryos of 11 mm. to 11.5 mm.) the proximal portion of the primitive ulnar lymphatic, now entirely emptied, separates from the jugular sac at a point a little caudal and dorsal to the course of the sixth spinal nerve (fig. 16, 10). The reconstructions of this period give the impression that the rapid increase in the size of the brachial plexus, situated ventral to the lymphatic channel, is a mechanical factor in interrupting the earlier connection of the lymphatic with the jugular sac.

In the following stages (12 mm. to 14 mm.) the cephalic portion of the primitive ulnar lymphatic continues to diminish in size. Its lumen becomes more and more reduced, the endothelial cells originally lining the channel become enlarged, stain deeply and gradually assume an irregular cuboidal form. Finally along the line of the former channel there remains only a clump of these deeply stained cells, reverted from the endothelial to the indifferent mesenchymal type, to indicate the site of this segment of the earlier primitive ulnar lymphatic (fig. 16, 10). In the meanwhile the distal portion of the primitive ulnar lymphatic, situ-

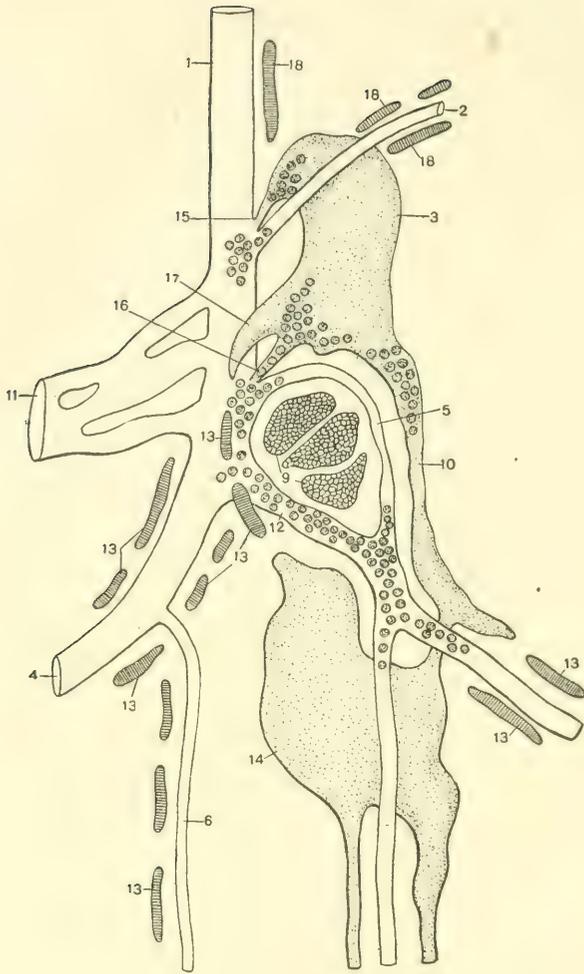


Figure 15

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|---|--|
| 1, Precardinal vein | 14, Subclavian or axillary lymphatic sinus |
| 2, External jugular-cephalic vein | 15, Common jugular lymphatico-venous tap |
| 3, Jugular lymphsac | 16, Jugulo-subclavian lymphatico-venous tap |
| 4, Duct of Cuvier | 17, Ventral prolongation from subclavian approach of jugular lymphsac to meet subclavian, axillary and mediastinal systemic lymphatics |
| 5, Primitive ulnar vein | 18, Jugular systemic lymphatic anlagen |
| 6, Postcardinal vein | |
| 9, Brachial plexus | |
| 10, Primitive ulnar lymphatic | |
| 11, Brachio-cephalic anastomosis | |
| 12, Subclavian vein | |
| 13, Anlagen of subclavian, axillary and mediastinal systemic lymphatics | |

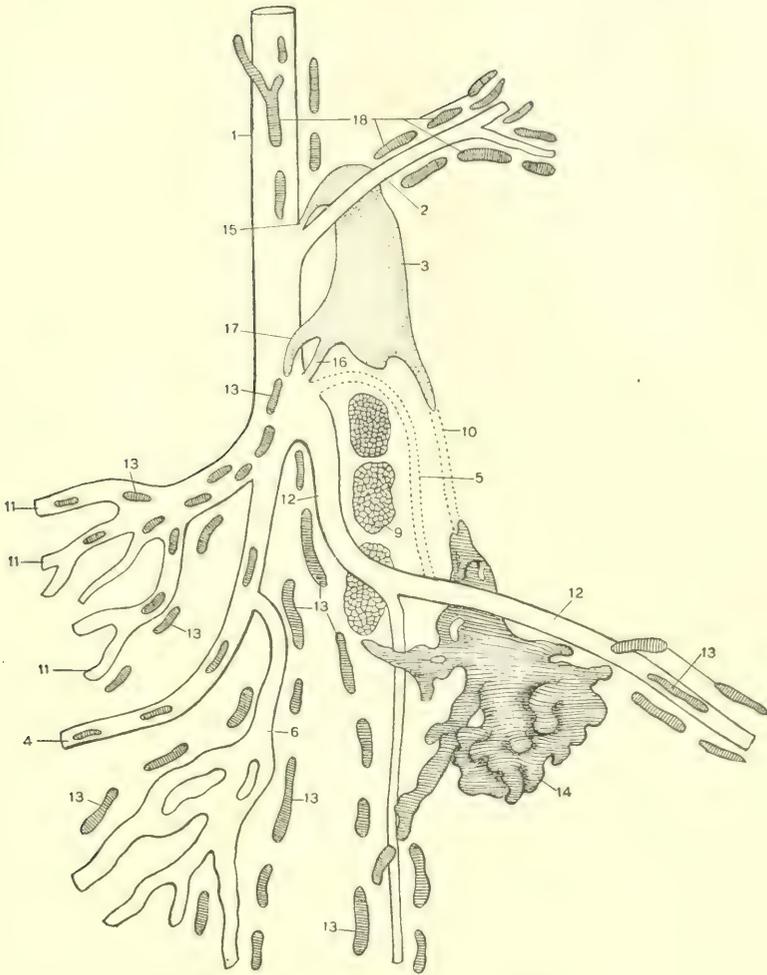


Figure 16

- | | |
|---|---|
| 1, Precardinal vein | 14, Subclavian or axillary lymphatic sinus |
| 2, External jugular-cephalic vein | 15, Common jugular lymphatico-venous tap |
| 3, Jugular lymphsac | 16, Jugulo-subclavian lymphatico-venous tap |
| 4, Duct of Cuvier | 17, Ventral prolongation from subclavian approach of jugular lymphsac to meet subclavian, axillary and mediastinal systemic lymphatics (13) |
| 5, Abandoned line of primitive ulnar vein | 18, Jugular systemic lymphatic anlagen |
| 6, Postcardinal vein | |
| 9, Brachial plexus | |
| 10, Abandoned line of primitive ulnar lymphatic | |
| 11, Brachio-cephalic anastomosis | |
| 12, Subclavian vein | |
| 13, Anlages of subclavian, axillary and mediastinal systemic lymphatics | |

ated caudal to the cords of the brachial plexus, enlarges rapidly and becomes distended with clear lymphatic fluid. In embryos between 14 mm. and 16 mm. the resulting axillary or subclavian lymphatic (14) bag attains relatively enormous proportions, often quite equaling the jugular sac in size. The intercellular mesenchymal spaces of this entire region likewise appear distended with clear fluid. The cells limiting these spaces become flattened and the entire mesenchyme presents the appearance of a multilocular spongy reticulum distended with fluid (fig. 16). Many of these mesenchymal spaces become connected with the axillary lymph bag and many others are actually incorporated in it, being largely responsible for its rapid and extreme increase in size. The axillary sac, having lost its early dorsal drainage through the primitive ulnar lymphatic into the jugular sac, now appears as a *closed reservoir* receiving the interstitial fluids of the body wall and anterior limb bud and storing the same temporarily.

In the meanwhile the primitive ulnar vein has likewise atrophied in its proximal cephalic segment (figs. 15 and 16, 5). The venous return from the trunk and anterior extremity is now carried by the newly established channel of the subclavian vein (12), situated caudal and ventral to the brachial plexus (9).

In this and the succeeding stages numerous isolated and independent perivenous mesenchymal spaces form along and around the subclavian vein (13). These, the anlagen of the future subclavian systemic lymphatics, then unite with each other into a rich perivenous lymphatic plexus which distally establishes connections with the axillary lymphatic reservoir. In a proximal direction they extend cephalad along the subclavian vein, until they finally unite in a plexiform connection with the ventral prolongation which extends from the subclavian approach of the jugular sac caudad over the ventral face of the jugulo-subclavian venous angle (17), and which also receives the lymphatic chain developed along the course of the internal mammary and thymic veins (fig. 17). As soon as the connections of the axillary sac with the jugular sac have been established through the subclavian lymphatics, the reservoir rapidly diminishes in size. This stage is usually reached in embryos of 21 mm. to 22 mm., but

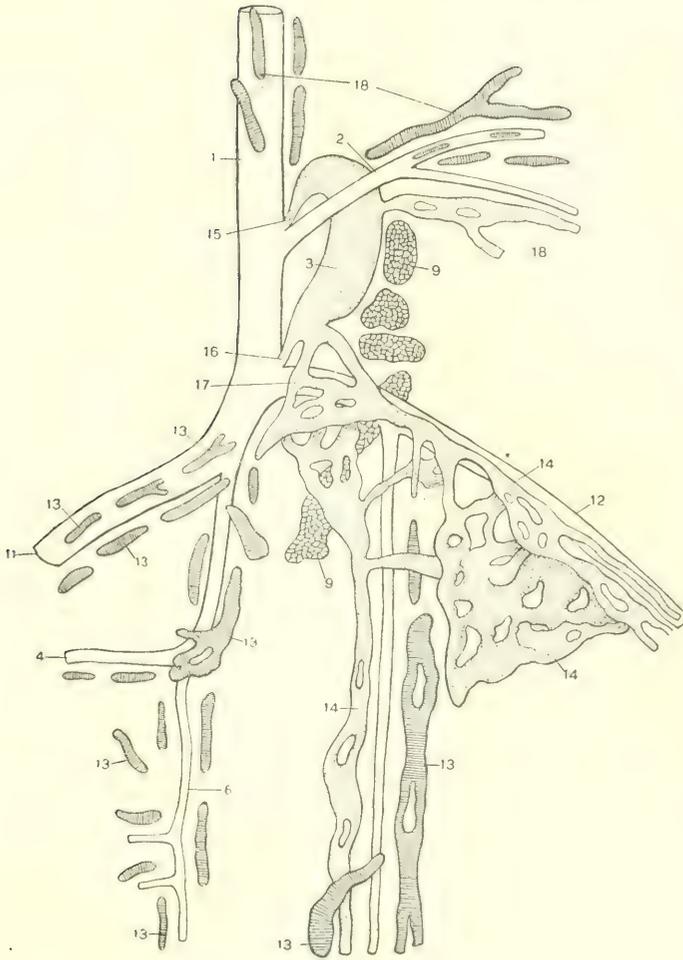


Figure 17

- | | |
|---|---|
| 1, Precardinal vein | 15, Common jugular lymphatico-venous tap |
| 2, External jugular-cephalic vein | 16, Jugulo-subclavian lymphatico-venous tap |
| 3, Jugular lymphsac | 17, Confluence of ventral prolongation from subclavian approach of jugular lymphsac with subclavian and axillary systemic lymphatics (14) |
| 4, Duct of Cuvier | 18, Jugular systemic lymphatic vessels and anlagen |
| 6, Postcardinal vein | |
| 9, Brachial plexus | |
| 11, Left brachio-cephalic vein | |
| 12, Subclavian vein | |
| 13, Anlagen of subclavian, axillary and mediastinal systemic lymphatics | |
| 14, Subclavian or axillary lymphatic sinus and connected subclavian and thoracic systemic lymphatic vessels | |

is often delayed until the 24 mm. or 25 mm. period. The deep lymphatics of the anterior limb-bud, developing relatively late, empty into the reduced axillary sac and attain their venous connection through the same and the proximal subclavian lymphatics uniting the axillary and jugular sacs (fig. 17).

This brief genetic history of the primitive ulnar lymphatic is, within the normal range of embryonal variation, repeated with remarkable consistency in a very large series of closely graded embryos of the domestic cat. The details, with the evidence presented by the sections and reconstructions, will shortly be published *in extenso* in one of the Memoirs of The Wistar Institute of Anatomy. •

For the purpose of the present communication it will suffice to generalize the following conclusions:

1. In certain regions lymphatic vessels develop during the early embryonal stages, primarily for the purpose of conveying the cellular products of the haemopoetic mesenchymal areas by direct channels into the venous circulation. Functionally these early lymphatic vessels are essentially *haemophoric*. During the period of this functional activity they offer no morphological criteria differentiating them from the adjacent haemal channels. Both the embryonal veins and the related haemophoric lymphatics are engaged in conveying red blood cells to the systemic venous circulation.

Much of the confusion of terms and of interpretation found in the records of the earlier investigations into the development of the lymphatic system is due to the misconception of this early functional character of these primitive lymphatics. They have, owing to their blood-cell contents, been classed indiscriminately as venous tributaries. In the course of further development these early haemophoric lymphatics may, after performing their primitive function, atrophy completely and disappear as components of the definite lymphatic system. This happens in the case of the proximal portion of the primitive ulnar lymphatic of the mammal, as above described. In other instances the early haemophoric lymphatics, after conveying the developing blood cells to their destination within the lumen of

the large veins, are retained as functional systemic lymphatic trunks in the permanent lymphatic organization.

Thus Miller (15) finds in embryos of the chick, in the axial preaortal line of the earlier stages, strands and masses of mesenchymal cells, which differentiate as developing blood cells *in situ* out of the indifferent mesenchymal syncytium. The anlagen of the thoracic duct arise, in close association with these strands of developing blood-islands, as "isolated lacunae directly from mesenchymal intercellular spaces, are not in any sense derived from the veins, and subsequently coalesce to form the continuous channels of the thoracic duct." Miller further states that the blood cells of the earlier axial strands gain access to the mesenchymal spaces constituting the anlagen of the thoracic duct, and that finally "the haemal cellular elements in question reach the blood-stream *via* the thoracic duct and jugular lymph-sac."

Miller's observations are of the utmost importance, because they reveal to us, for the first time, the main lymph channel (thoracic duct) of an amniote vertebrate in the *haemophoric stage*, and establish this stage as a potential type in the lymphatic ontogeny of any vertebrate embryo. Miller has, in other words, removed the greatest obstacle we have heretofore encountered in our attempts to establish a correct definition of developing lymphatics in contrast to developing veins. His results prove, I believe, conclusively that in certain vertebrate embryos and in certain stages *lymphatic* vessels may be actively concerned in transporting blood cells to the general haemal circulation, and that during this process these *haemophoric* lymphatics, still isolated from the general complex of the lymphatic system as a whole, have been mistakenly regarded as venous derivatives and cannot, as a matter of fact, be distinguished, in isolated sections and without reconstruction, from true venous tributaries. I regard this discovery of Miller's and the resultant correction of Sala's earlier work ('00) (24) in the same field, as the most important contribution to the elucidation of the lymphatic problem which has yet been made.

It is now possible to directly compare conditions observed in embryos of the different vertebrate classes during the development of the lymphatic system, without being led astray by the signal divergences encountered. Thus Miller's account of the development of the avian thoracic duct repeats, on a large and impressive scale, the above described stages in the development of the mammalian jugular sacs and primitive ulnar lymphatics. In the further course of development the early haemophoric axial lymphatics of the bird are *retained* in the permanent lymphatic organization as the main systemic lymphatic channels of the thoracic ducts. In the mammal the longer cephalic segment of the primitive ulnar channel, after fulfilling its earlier haemophoric function, *atrophies* and *disappears*, while the distal portion, becoming the axillary lymphatic sac, serves at first as an extensive temporary lymphatic reservoir, and then becomes incorporated in the system of the permanent lymphatic drainage of the body wall and anterior limb.

2. A second fact, which appears to me to be of great physiological and structural importance, is the establishment, in certain areas of the embryo, of large temporary lymphatic receiving chambers or reservoirs, in which the interstitial fluids of the developing embryo are apparently stored pending the completion of lymphatic channels and connections through which this fluid is eventually turned into the plasma-stream of the venous circulation.

The axillary or subclavian reservoir above described is a very pregnant instance of this condition. Its early connection, dorsal to the brachial plexus, with the jugular lymphsac during the haemophoric period, the subsequent interruption of this communication, the ensuing enormous fluid distension of the sac, and finally its secondary drainage through the chain of subclavian lymphatics developed caudoventral to the brachial plexus, are all definite and constant phases which in combination with each other furnish a very clear and significant picture.

In part these lymphatic ontogenetic stages are without doubt closely associated with and dependent upon the rearrangement of the main venous lines of the neck, thorax and anterior limb,

which finally result in the substitution of the subelavian for the primitive ulnar vein.

In part, as above stated, the development of the massive cords of the brachial plexus appears to exert an important mechanical influence on the rearrangement of the lymphatic system. This factor seems to render the early route of the circulation through the primitive ulnar vein and lymphatic, *dorsal* to the developing plexus, more difficult to maintain, and favors the secondary development of both venous and lymphatic channels along the easier path of the subelavian line, *caudal* and *ventral* to the nervous plexus.

3. Finally, comparison of lymphatic development of *reptilian* with *avian* and *mammalian* embryos shows a remarkable correspondence in the basic genetic processes. In reptiles both the jugular lymphsacs and the systemic lymphatic channels are developed along the same lines as in birds and mammals (12, 14). While this general agreement in the fundamental principles governing the development of both the haemal and the lymphatic divisions of the vascular system obtains in all the three amniote classes, the reptilian embryo exhibits certain special characters, when contrasted in detail with the mammal and the bird. These differences are founded on the functional and structural *adaptions* of the genetic ground plan common to all amniotes to the specialized physiological and metabolic conditions presented by the reptile.

In detail the reptile is characterized by the relatively enormous unfolding of the systemic lymphatic channels and sinuses. Their genesis is identical with that observed in mammalian and avian embryos, but they obtain a great preponderance in size and extent over the blood-vascular channels. I presume that this is the morphological expression of the lowered combustion-coefficient of the latter in cold-blooded vertebrates. In all three amniote classes the basic principle active in the development of the systemic lymphatic vessels is the formation of independent intercellular mesenchymal spaces, with resulting independent origin of the lymphatic endothelium by direct modification of the mesenchymal cells limiting the early spaces.

In the reptilian embryo the greater number of the important systemic lymphatic sinuses, produced by the fusion of these earlier anlagen, develop without any relation whatsoever to the embryonal veins, as independent periarterial channels. Consequently in the reptilian embryo the question of their direct or indirect origin from the veins never even arises. Only a small number of the reptilian systemic lymphatics follows the embryonal veins, surround them and in some instances replace early venous lines.

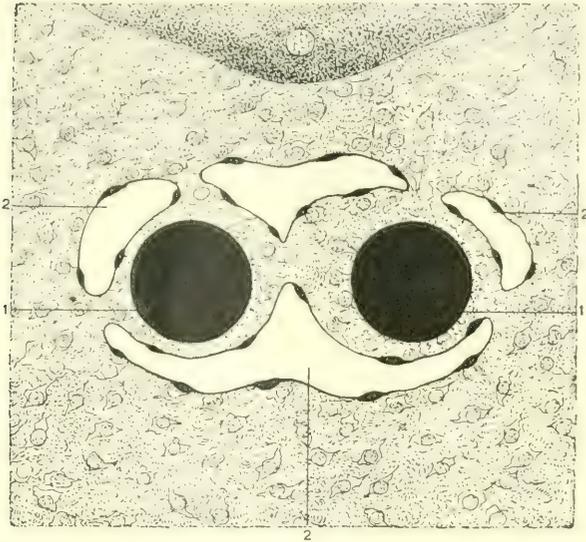
The early anlagen of the reptilian jugular lymphsacs are in my opinion without doubt *primitive haemophoric lymphatics* in the sense above defined for the homologous anlagen of the mammalian jugular sacs (*vide supra*, pp. 273-280).

This is especially true for the element which I described in the publication previously quoted (12) as the posterior or caudal division of the ventral veno-lymphatic sinus in lacertilian embryos.

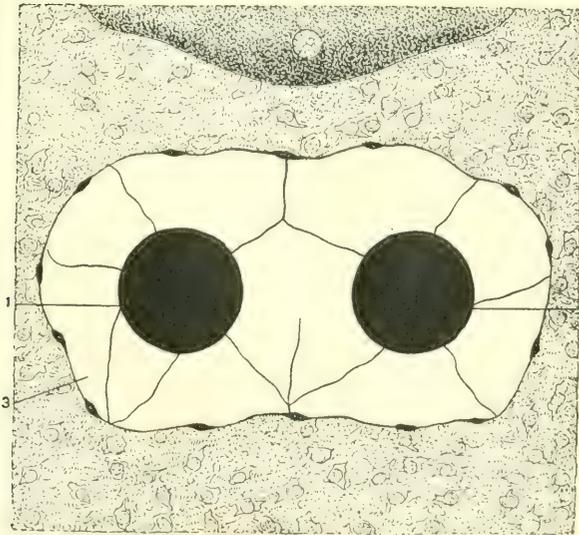
For the purpose of comparing the development of the systemic lymphatic system in embryos of the three amniote classes it is perhaps best to contrast the typical ontogenetic stages in a single and definite area in each. The region of the main axial systemic lymphatic channels (thoracic ducts) are thus contrasted in the series of diagrams shown in figures 18 to 20.

1. In the reptile (chelonian and lacertilian embryos²) the large adult periaortal lymphatic sinuses develop at first as small intercellular clefts in the spongy mesenchyme surrounding the dorsal aortic arches and their caudal prolongation as the single trunk of the dorsal aorta (fig. 18, A, 2). These spaces enlarge, approach each other, fuse and finally surround the aorta as a huge periarterial lymphatic sinus, with trabeculae in the interior, representing remnants of the original partitions between the components of the sac (fig. 18, B, 3). This extensive periaortal sinus represents in Lacertilia and Chelonia the much reduced thoracic ducts of birds and mammals. It establishes secondary connec-

² *Aspidonecetes spinifer*; *Chelydra serpentina*; *Chrysemys marginata*; *Chrysemys picta*; *Pseudemys scripta*; *Cnemidophorus sexlineatus*; *Sceloporus undulatus*.



18 A



18 B

Figure 18A and 18B

1, Dorsal aortae

2, Anlages of periaortic lymphatics

3, Periaortic lymphatic sinus (coelomic duct)

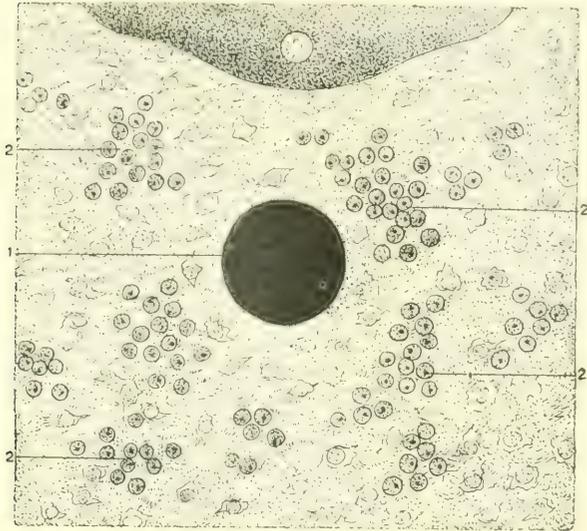
tions with independently developed peripheral lymphatic channels, joins the jugular lymphsacs and through them attains its entry into the venous system.

From its earliest inception the reptilian periaortic sinus is at no point in relation to the venous system. It is intimately related to the dorsal aortae, but there are, at the site of its development, no large embryonal venous channels corresponding to the mammalian azygos (post- and supracardinal) trunks. Consequently the developing thoracic, or rather coelomic lymphatic sinuses of the reptile never come into intimate genetic or topographical relations with axial veins.

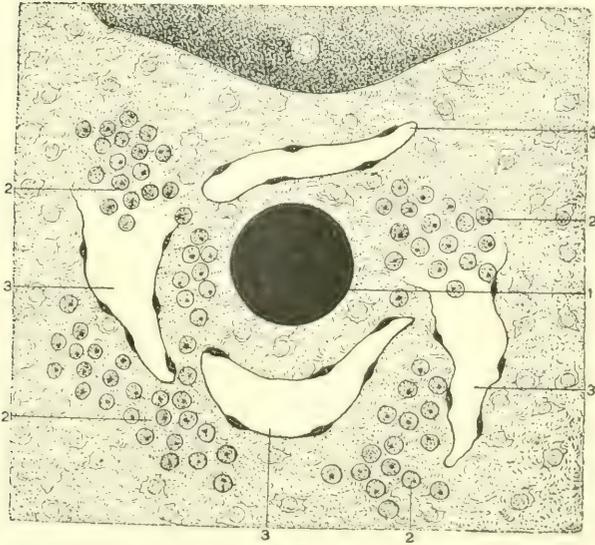
Further, the axial periaortic mesenchyme of the reptilian embryo is not the site of an active haemopoiesis. Consequently, in strong contrast with the avian type, the reptilian homologues of the thoracic ducts never become *haemophoric*.

The reptile offers hence in this region the clearest and least complicated illustration of the basic principle underlying *all* vertebrate angiogenesis in general and all lymphatic ontogeny in particular, viz., the formation of a system of connected channels, developed by fusion of originally separate and independent intercellular mesenchymal spaces, not complicated by any relation whatsoever to the systemic veins (fig. 18, *B*), nor charged with the haemophoric function of conveying red blood-cells developed *in situ* into the general haemal circulation. The bird (fig. 19) follows the general reptilian type of development with the following important modifications:

The periaortal mesenchyme of the chick is the site of a most active and abundant intraembryonic haemopoiesis *in situ*. Masses of developing blood cells differentiate as axial strands [the 'mesenchymal cords' of Sala (24)] around the aorta, directly from the indifferent periaortic mesenchymal syncytium (fig. 19, *A*, 2). Subsequently the anlagen of the thoracic ducts appear in this periaortic area as isolated intercellular mesenchymal clefts and spaces (fig. 19, *B*, 3). These spaces become confluent, receive the blood cells developed in the mesenchymal blood-islands, and convey them through the channels of the thoracic ducts to the jugular lymphsacs, and through them into the circulating venous



19 A



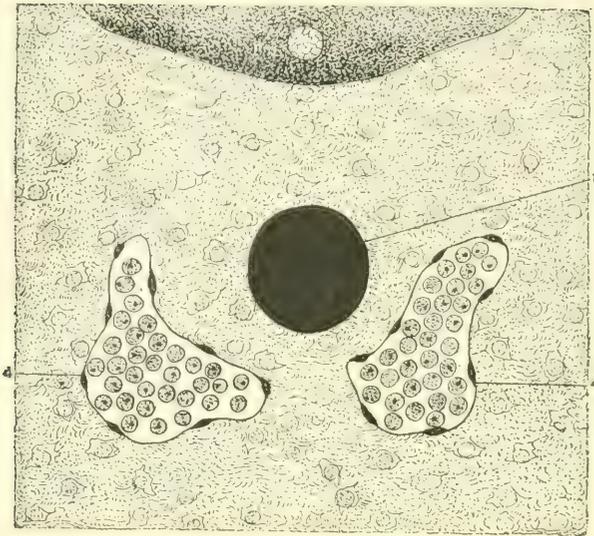
19 B

Figures 19 A and 19 B

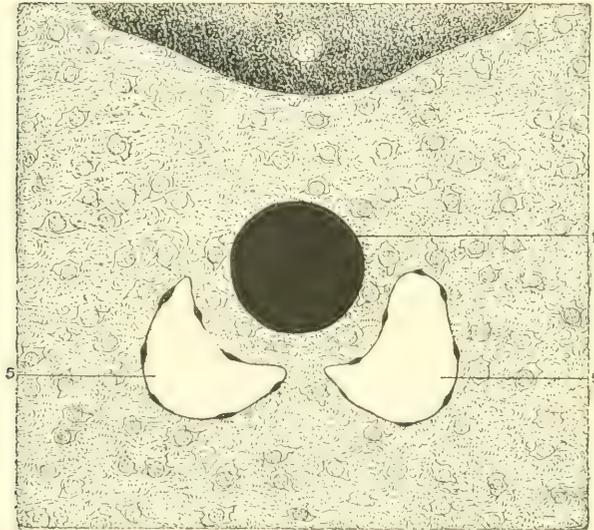
1, Aorta

2, Periaortic blood islands

3, Periaortic mesenchymal lymphatic spaces, the anlages of the thoracic ducts



19 C



19 D

Figures 19 C and 19 D

1, Aorta

4, Thoracic ducts, haemophoric stage

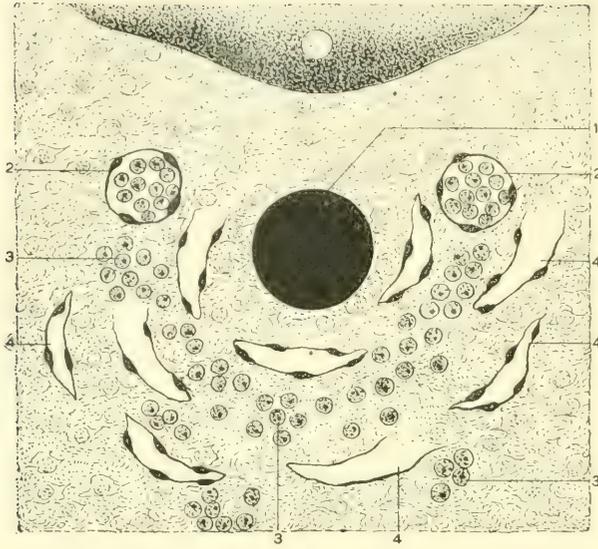
5, Thoracic ducts, evacuated

stream (fig. 19, *C*, 4). After this evacuation of their early blood contents the axial lymphatic channels are retained as the permanent avian thoracic ducts (fig. 19, *D*, 5). The bird, therefore, in comparison with the reptile, presents identical morphogenetic characters, only differing in the degree of their development. The reptile unfolds the simple periaortic lymphatic channels by confluence of innumerable independently developed mesenchymal spaces. The bird follows the same genetic plan, but the intercellular mesenchymal spaces at once come into close relation with the periaortic haemopoietic mesenchyme which is not developed in the reptile. Hence in the early phase the avian thoracic duct becomes a haemophoric lymphatic channel.

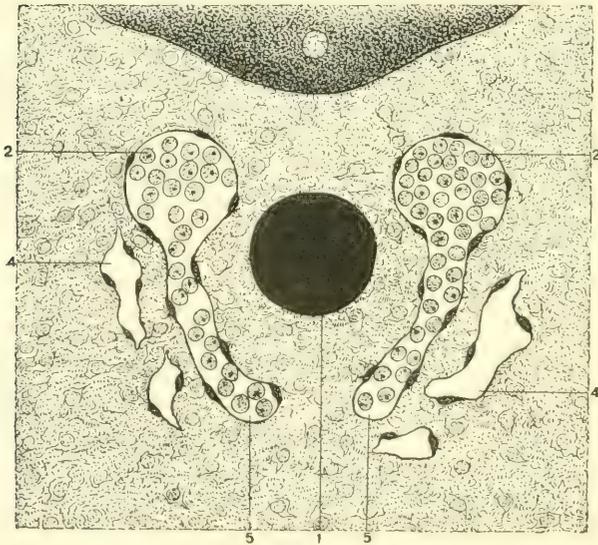
In the mammal (fig. 20), as shown by a number of recent investigations, the anlagen of the thoracic ducts develop as independent intercellular mesenchymal spaces in close association with the temporary ventro-medial tributary plexus of the azygos veins (fig. 20, *A* and *B*, 4). Subsequently these venous radicles are surrounded by the growing lymphatic spaces (fig. 20, *C*, 6), become detached from the azygos veins, atrophy, and are finally *replaced topographically* by the thoracic ducts (fig. 20, *D*, 7).

This mode of lymphatic development has been described by McClure and myself as the 'extra-intimal type,' because the lumen of the lymphatic anlage is always *outside* of the intimal lining of the degenerating vein which the resulting lymphatic channel is destined to replace.

The mammalian embryo, compared with the embryos of the two remaining amniote classes, offers specialized conditions in the axial region of the trunk by reason of the presence of the azygos or supracardinal system of veins. Differentiation of mesenchyme *in situ* into developing blood cells occurs in this region as it does in avian embryos (fig. 20, *A*, 3). The products of this haemopoiesis are, however, conveyed in the mammal directly into the azygos veins through the temporary ventro-medial tributary plexus (fig. 20, *B*, 5). This plexus atrophies after performing this haemophoric function, and is topographically replaced by the thoracic ducts (fig. 20, *C*, 6; fig. 20, *D*, 7). In the bird, on the other hand, the absence of axial veins in this area



20 A

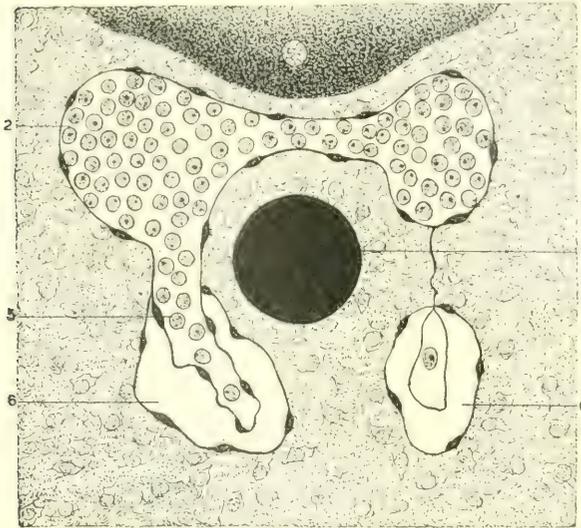


20 B

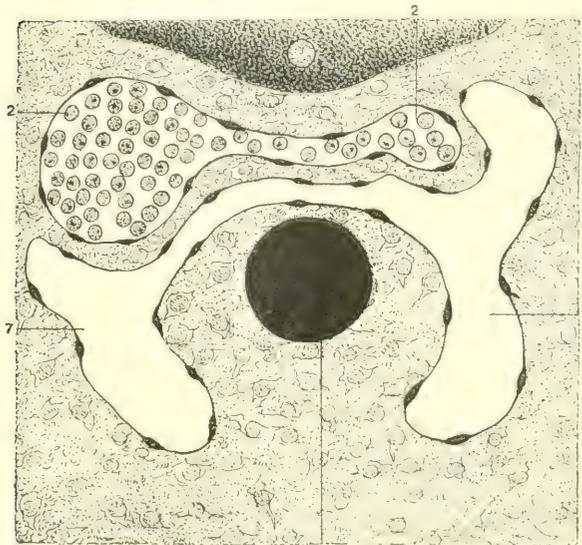
Figures 20 A and 20 B

- 1, Aorta
- 2, Azygos veins
- 3, Periaortic blood-islands

- 4, Periaortic mesenchymal lymphatic spaces
- 5, Temporary ventro-medial azygos tributary plexus



20 C



20 D

Figures 20 C and 20 D

- | | |
|--|--|
| <p>1, Aorta
2, Azygos veins
5, Temporary ventro-medial azygos tributary plexus</p> | <p>6, Extra-intimal perivenous lymphatic spaces, the anlagen of the thoracic ducts
7, Thoracic ducts</p> |
|--|--|

imposes on the developing thoracic ducts the additional function of conveying the newly developed blood cells to the venous circulation.

It will thus be seen that the development of the thoracic ducts in all three amniote classes follows precisely the same main fundamental principle, viz., the formation of lymphatic channels by confluence of numerous originally separate intercellular mesenchymal clefts and spaces.

The reptile presents this genetic process in its simplest form, in a region in which systemic venous development is reduced to a minimum. In both the bird and the mammal the development of the thoracic duct becomes complicated by a direct or indirect relation of the lymphatic anlagen to the adjacent elements of the haemal (venous) vascular system. In the bird the relation of the lymphatic anlagen to the haemopoetic axial mesenchyme is *direct*, the avian thoracic ducts becoming for a time functionally *haemophoric*.

In the mammalian embryo the relation of the developing thoracic ducts to the axial venous system is *indirect*, the lymphatic anlagen *replacing* the temporary ventro-medial haemophoric azygos tributaries *topographically*, but never themselves assuming the haemophoric function.

In all three classes of amniote embryos the final result of the genetic processes outlined above is the same, viz., the establishment of a periaortic, or paraaortic, lymphatic channel, the amniote thoracic duct. In a wider interpretation it becomes evident that all the diversified phenomena of vascular ontogeny, haemal as well as lymphatic, are focussed in the small field which any extra-embryonic vascular area presents. These phenomena comprise:

A. Blood-vascular development

1. Development of intercellular mesenchymal spaces.
2. Confluence of the same to form connected channels.
3. Modification of the mesodermal cell lining the spaces and channels as the vascular endothelial cell.
4. Aggregation of mesenchymal cells into vascular strands and islands in which active haemopoiesis takes place.

5. Entrance of these developing blood cells, after resolution of the islands, into the above described spaces and channels, and the incorporation of the free cells in the haemal circulation.

B. Development of the non-haemophoric systemic lymphatic vessels.

The development of intercellular mesenchymal spaces, their confluence to form a connected system of channels, the modification of the mesodermal parietal cells as lymphatic endothelium, and the establishment of secondary connections between this lymphatic system and the veins. All these genetic processes are in principle identical with the primary stages of the blood-vascular development, prior to the inclusion of the free blood cells in the haemal channels (A, 1, 2 and 3).

C. Development of the haemophoric or veno-lymphatic portion of the lymphatic system

This proceeds on lines identical with the haemal vascular development up to the point where the free blood cells are added to the circulating venous stream.

After this evacuation the early haemophoric lymphatic channels or sacs revert to the lymphatic system proper in whole or in part. The distribution of the haemophoric division of the general lymphatic system varies in the three amniote classes. In the mammalian and reptilian embryo the haemophoric lymphatic area of the anterior portion of the body is chiefly represented by the elements of the jugular lymphsac and by the primitive lymphatic drainage of the anterior limb-bud (primitive ulnar lymphatic).

In the avian embryo, addition to the jugular lymphsac, the entire axial lymphatic line of the thoracic ducts is haemophoric in the primitive stages.

In all three classes the jugular lymphsacs, developed from early haemophoric lymphatic anlagen, serve, after performing this function, as the anterior links or junctional segments between the veins and the vastly more extensive complex of the non-

haemophoric systemic lymphatics developed independently by confluence of intercellular mesenchymal spaces.

In the foregoing analysis it should be kept in mind that the term 'haemophoric lymphatic' is used solely to define the temporary embryonal stage in which, in certain embryos, newly developed blood cells which arise *in situ* in the mesenchymal syncytium and which have *never before circulated*, are conveyed by lymphatic vessels into the circulating venous stream. The question of the reversed passage of blood which has *once circulated* from the veins into lymphatic vessels and sacs in open secondary communication with the veins, prior to the development of the lymphatico-venous valves, is not here considered.

In the same way the present paper does not touch on the question of the development of erythrocytes from the endothelium lining the early lymphatic channels. It is possible that this takes place in late embryonal or even postnatal stages, and some of the structures described as haemolymph nodes may owe their existence to such a prolonged haemopoiesis.

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THE SKULL OF A HUMAN FETUS OF 40 MM.

CHARLES CLIFFORD MACKLIN

James H. Richardson Fellow in Anatomy, University of Toronto

EIGHTEEN FIGURES

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INTRODUCTION

Researches upon the developing human skull have been carried on by various investigators, for a summary of whose results reference may be made to the excellent reviews by Jacoby ('94) and Levi ('00). The plate method of reconstruction, however, has been applied in such investigations only within recent years, and has been used in the study of fetuses in the following stages:

LENGTH	PROBABLE AGE (AUTHOR'S ESTIMATE)	AUTHOR
<i>mm.</i>		
13		Levi
14	• 37-38 days	Levi
17	42-45 days	Levi
17	50 days	v. Noorden
18.5	7½ weeks	v. Noorden
23	8½ weeks	v. Noorden
30		Jacoby (younger than the 28 mm. stage of Levi)
28	58-62 days	Levi
30		Fawcett
80		Hertwig

It will be seen that a considerable interval occurs between the last two stages in this list, and with a view towards lessening it I undertook, at the suggestion of Professor McMurrich, the study of the skull of a fetus of 40 mm., crown-rump measurement, which would correspond, according to Mall's formula (Mall '06, p. 439), to an age of 63 days. The entire head of the fetus had been cut into serial sections in the frontal plane, the sections being 20 micra in thickness and stained with haemalum followed by erythrosin. From these sections the entire skull, together with the membrane bones and upper two visceral arches, was reconstructed by the wax plate method at an enlargement of 30 diameters, and, to facilitate the study of the nasal and otic capsules, separate models were made, at the same magnification, of the mesethmoid, ectethmoid, right otic capsule and the anlage of the right osseous labyrinth. Drawings were made of every other section by the use of Edinger's "Drawing and projection apparatus," only that tissue being included as cartilage which presented a homogeneous, blue ground-substance and could be clearly differentiated from its surroundings, i.e., the tissue which Levi ('00) has described under the terms 'alteren Vorknorpel' or older precartilage, and "Knorpel" or cartilage. The earlier chondrogenic tissue of which there was very little at this stage, was disregarded.

The sections were not exactly in the frontal plane and to eliminate any lateral distortion in the models the amount of obliq-

uity was determined as accurately as possible and the wax plates piled upon a plane adjusted at the proper inclination. It is also to be noted that a few sections passing through the dorsal extremity of the occiput were wanting from the series, and the small portion of the occipital region which they represented has been reconstructed by reference to other models, the parts so added being painted white, so that they may readily be recognized.

In the following description of the models I have used, as far as possible, the terms which are current in the literature, and when those referring to the human skull were exhausted recourse was had to the terminology in use in the more recent publications upon the chondrocrania of mammals. Preference is given to the BNA, and names not hitherto introduced into human chondrocraniology are usually followed by the name of the author who has employed them, the ideal being always kept in view of a system of terms uniform throughout the mammalian forms at least. New names, introduced by the writer, are indicated by making their initial appearance in italics.

An attempt has been made to select such terms of orientation as may be applied to animals having either a horizontal or a vertical long axis. Thus the terms ventral, dorsal, cranial, caudal, lateral and medial are generally used, but their respective equivalents for the human figure, such as anterior or front, posterior or back, superior or upper, inferior or lower, external or outer, internal or inner, are also employed. Oblique directions are indicated by combinations of the above terms.

Measurements appearing in the text have been taken from the models, and are thus magnified thirty times.

THE SKULL AS A WHOLE

The primitive skull of homo at the 40 mm. stage presents, in general, the characters which have become familiar through the illustrations and descriptions of v. Noorden, Jacoby and Levi for younger embryos, and the model of Hertwig for a more advanced age, combined with several features that are characteristic of this period of development. The outline, when the many gaps are filled in, suggests the osseous skull.

Viewed from above (fig. 1) we note the entire absence of the roof and the extremely rudimentary character of the sides of the cranial vault. The eye meets with an irregular surface of varying depth, surrounded by a broken, ovoid contour, the smaller end being ventral. This surface we recognize as the floor of the primitive brain-case. Its dorsal half is made up of the future posterior cranial fossa, a deep, bowl-like enclosure, the steep sides of which slope rapidly down to an elongated opening in the floor—the primitive foramen magnum. In the ventral wall of this fossa is a trough-like space behind the basilar plate, flanked by two rounded eminences, the partes cochleares of the otic capsule, and terminated above by the horned, ridge-like dorsum sellae, which forms a conspicuous object in the floor of the cranium. Passing forward over this ridge a sideless pit, the hypophysial fossa, comes into view, which marks the center of the middle cranial fossa, and here, in the region of the body of the sphenoid, the cartilaginous floor is very narrow. Lateral to the corpus sphenoidale is a large, triangular gap in the floor and sidewall of the brain-box, the apex of which meets the side of the sella, while the ventral and dorsal borders are formed by the dorsal border of the ala orbitalis and the cranio-ventral surface of the otic capsule respectively. Forming a lateral, knobbed projection beneath the ala orbitalis is the relatively small ala temporalis, and this is observed to lie ventro-caudal to the plane of the above-mentioned triangular space, just as, in the osseous skull, the greater wing lies below and in front of the plane joining the lesser wing with the petrous portion of the temporal bone. As will be seen later, this plane corresponds in a general way to the situation of a primitive floor and side-wall of this region of the skull, as found in the lizards. Two of the bones which will later wall in this space, viz., the parietal and the squamo-temporalis, are as yet very rudimentary, while the third, or ala temporalis, has only just commenced to ossify.

We now pass forward, over the low ridge in front of the sella turcica, known as the tuberculum sellae, and come upon the plateau-like surface supporting the optic chiasma, which leads laterally into the optic foramina. Ventral to this surface is the

wide, upward-slanting, anterior root of the ala orbitalis, in front of which is a broad, flattened, triangular surface. This we recognize as the floor of the anterior cranial fossa. Perforating it, in the area lateral to the median septum, which represents the developing cribriform plate, are several foramina, the largest being the paired fenestrae cribrosae. Hook-like, backwardly projecting processes mark the dorso-lateral limits of the floor, which ventro-laterally is widened by the orbital plate of the frontal bone.

The only portions of the osseous cranial vault yet in evidence are the rudimentary frontal portion of the frontal bone and the net-like parietal—these being separated by a rather wide space.

Finally, the cranial aspect discloses to view certain accessory cartilages. Above the dorsal wall of the posterior cranial fossa may be seen two cartilages, lying close together, both small, but the left considerably smaller than the right. They may be known as the *cartilagine cranii posteriores*. Above each parietal plate, medial to the parietal bone, is seen an isolated nodule of cartilage, that on the right being larger and more elongated than that on the left; these may be called the *cartilagine cranii laterales*. Below and in front of the cranial pole of each cochlea there is a small, rounded mass of cartilage, which may be termed the *cartilago supracochlearis*.

CARTILAGE

Passing, next, to a consideration of the lateral aspect of the primitive skull (fig. 3), we note that it is made up of two main masses, dorsal (posterior) and ventral (anterior), almost separated by a large gap between the otic and orbitotemporal regions. The dorsal region is composed principally of cartilage, and presents a fairly smooth, convex, fenestrated surface, the most lateral part being formed by the rounded outer wall of the pars canalicularis of the otic capsule. Behind this the flat surface is seen to widen, and then quickly narrow, and to sweep backwards and inwards to unite with the corresponding plate of the opposite side in the tectum posterius. Above, the wall is heightened

by the reticular parietal bone (fig. 4). These structures enclose the lower and posterior portion of the cranial cavity.

The anterior mass is composed principally of the facial parts, and contrasts with the posterior in being narrower from side to side in its lower part, and in presenting a greater proportion of osseous material. Its surface is also much more irregular and uneven. In front of the *pars canalicularis* we see, in a recess which lies ventro-lateral to the two parts of the otic capsule, an irregular mass of cartilaginous and osseous structures, in which we recognize the anlagen of the auditory ossicles and the upper part of Meckel's cartilage, the latter appearing above the small tympanic and goniale (fig. 2), and being covered laterally by the squamo-temporalis. Below these structures, with its hook-like proximal extremity lying in a small cavity, but unconnected with the rest of the chondrocranium, is the slender shaft of Reichert's cartilage.

Above the otic capsule the large open space which will later form the middle cranial fossa is again apparent, and through this we see the lateral aspect of the hypophyseal fossa (fig. 3), with the high dorsum sellae, upon which are the ventrally projecting posterior clinoid processes, limiting it posteriorly. In the anterior wall of the sella turcica is the middle clinoid process (present on the right side only) and above this is to be seen the orbital wing of the sphenoid, the gentle curvature of the latter being broken by the anterior clinoid process, and its outer and posterior extremity terminated by a sharp process, projecting dorsally. Below the *ala orbitalis*, and separated from it by the wide and unclosed superior orbital fissure, is the *ala temporalis*, supported by a short *processus alaris*, the rounded extremity of the latter almost touching the ventral pole of the cochlea, and being quite close to the *cartilago supracochlearis*, which is plainly visible from the side.

Looking at the skull from a more anterior position we see, above, the cavity of the orbit, roofed over by the cartilage of the orbital wing of the sphenoid, and by the orbital portion of the frontal bone (fig. 4); limited medially by the shelving posterior portion of the ectethmoid; but widely open downwards and out-

wards, except where cut off by the zygomatic bone and the maxilla, and but imperfectly closed behind. In the posterior portion of the orbit is seen the elongated optic foramen, and the closeness of apposition of the sphenoidal and ectethmoidal cartilages is to be observed. An open space, communicating freely above with the orbit, below with the cavity of the mouth and medially with that of the pharynx, is seen in front of the ala temporalis, the medial wall of the space being indicated by the imperfectly developed vertical plate of the palatine bone and by the rudimentary medial pterygoid plate. The position of the as yet unformed pterygomaxillary fossa is indicated in this space by the sphenopalatine ganglion (fig. 14) (not shown in the model), and lateral to this, bounded by the incomplete zygomatic arch, are the temporal and zygomatic fossae. One is struck with the lack of prominence of the zygomatic region when compared with the osseous skull, the zygomatic bone and arch being completely overhung by the lateral part of the cranial floor. That this disproportion is partly due to the shallowness of the temporal and zygomatic fossae is evident from a comparison of the model with the bony skull. It would appear that the lateral growth of the ala temporalis of the sphenoid and of the zygomatic process of the maxilla, combined with thickening of the temporalis muscle, are the principal factors which bring about the widening of the temporal and zygomatic fossae, and consequent outpushing of the lateral parts of this area. It is evident from a comparison of the skulls of the newborn and the adult that this change continues till some time after birth.

The lower part of the facial region is characterized by the gaping cavity of the mouth, bounded above by the superior maxilla and below by Meckel's cartilage with its covering bone, the mandible (fig. 4). The lack of prominence of the angle of the latter, due to the shortness and inclination of the ramus and its relative nearness to the medial sagittal plane, may be observed, and it will also be seen that there is a small space between the articular process of the mandible and the position of the future glenoid cavity of the squamo-temporalis (fig. 4). The coronoid process of the mandible is quite close to the ala temporalis of the

sphenoid, medially, and to the zygomatic arch (as yet incomplete) laterally, a condition which does not obtain in the mature skull.

Dorsal to the prominent frontal process of the superior maxilla the isolated nodule of cartilage, known as the cartilago paranasalis, is to be seen, lying lateral to the cavity for the lacrimal duct and below the small streak of membrane bone, which is the anlage of the future lacrimal. Another smaller nodule of cartilage is seen near the back of the orbit lying against the upper surface of the ectethmoid and may be known as the *cartilago paraethmoidalis*. Separating the orbital from the nasal cavity is to be seen the shell-like ectethmoid, bearing upon its cranio-ventral aspect the small nasal bone. At the front of the nasal cartilages appear the open anterior nares, separated by the ventral border of the septum.

Regarded from below (fig. 2) the model shows in the foreground the mandibular (not shown in fig. 2) and upper part of the hyoid arches. Behind these we see the semi-cylindrical ventral surface of the planum basale, separated from the elongated, flattened, ovoid partes cochleares by deep furrows. It is to be observed that the anterior extremities of the latter do not project beyond the planum. The forked structure at the root of the planum, perforated for the hypoglossal nerve, is seen to be the anterior commencement of the flattened, ring-like sides of the foramen magnum, a downwardly projecting angle marking the position of the future occipital condyle. Lateral to and above the condyle there appears a stout cartilaginous process, which supports the lower and anterior part of the otic capsule. This is known as the processus paracondyloideus (Voit), and above it is seen a wide opening, the jugular foramen (fig. 4).

The two Meckelian cartilages (fig. 3) enclose an angle, rather sharp ventrally, in which are found the structures of the floor of the mouth. The inwardly curved palatine bones, with the assistance of the inner laminae of the pterygoid processes (fig. 2), imperfectly cut off the posterior part of the nasal cavity, and between the pterygoid laminae and the planum basale is seen the space occupied by the naso-pharynx (fig. 10). Attached to these medial laminae, and indeed developed from their caudal tips, are

the cartilagine parasphenoidales (Voit, '09), the representatives of the later hamular processes. The pterygoid laminae are quite separate from the alae temporales, which appear, from this viewpoint, as rhomboidal, perforated blocks.

Lying along the caudal border of the nasal septum are seen the anterior paraseptal cartilages in front, and the long thin plates of the vomer behind (fig. 2); and within each nasal cavity is a small mass of cartilage, lying in the middle meatus (fig. 12), to which the name *cartilago meatus medii* has been given and whose significance will be discussed with the regio ethmoidalis.

Summing up the cartilaginous and osseous structures which we find participating in the formation of the primitive skull, we have to consider a main cartilaginous mass, or chondrocranium, a number of accessory cartilages, the upper visceral arches, and the membrane bones.

The chondrocranium is a complicated mass of cartilage of exceedingly irregular formation, in which a number of definite areas may be recognized. Upon examination it is seen to consist of a larger dorsal and a smaller ventral enlargement, united by an isthmuslike part, the body of the sphenoid. A median stem, bent to enclose an angle of 115° open caudo-ventrally, forms the main axis, this being made up dorsally of the flattened planum basale and ventrally of the interorbital and nasal septa, or, employing the terms of Kölliker, the pars chordalis and the pars praechordalis respectively. These limbs are united by the corpus sphenoidale, or Balkenplatte of the German authors, which forms the apex of the angle. The four primary regions of the chondrocranium, which Gaupp has named, from behind forwards, the regio occipitalis, the regio otica, the regio orbitotemporalis and the regio ethmoidalis, are all represented in the median stem, in the order named, the first two being found in the pars chordalis and the second two in the pars praechordalis.

Springing out from the sides of the planum we have the walls of the posterior cranial fossa, while to the ventral end of the axis are affixed the side parts of the ethmoidal and orbitotemporal regions.

If the structures lateral to the ventral limb of this stem be removed, we have an object which roughly resembles a dipper or saucepan, the curved handle being made up of the ventral part of the stem, while the bowl, perforated below and at the sides, is formed ventrally by the planum basale, and laterally and dorsally by the walls of the posterior cranial fossa, the median lip being situated at the tectum posterius.

The chondrocranium is, at this stage, a continuous morphological unit, but there is histological evidence going to show that certain of its parts were primarily separate.

In addition to the chondrocranium proper a number of accessory cartilages, which have no direct connection with it and which have already been mentioned, also occur. They will be discussed in connection with the regions to which they refer, and are as follows:

Cartilago cranii posterior.....	Regio otica
Cartilago cranii lateralis.....	Regio otica
Cartilago supracochlearis.....	Regio otica
Cartilago parasphenoidalis.....	Regio orbitotemporalis
Cartilago paraethmoidalis.....	Regio ethmoidalis
Cartilago paranasalis.....	Regio ethmoidalis
Cartilago meatus medii.....	Regio ethmoidalis

Of the recorded human embryonic skulls that of Levi ('00) for the 28 mm. stage most closely resembles my specimen, but is somewhat younger. The next nearest stage, represented in the Ziegler model of Hertwig's 80 mm. embryo, is somewhat older.

The principal change which has occurred in the interval between the 28 and 40 mm. stages appears to be the development of the anterior cranial fossa. In the Levi specimen this is relatively narrower and deeper than in mine, thus indicating an adaptation of this region to the increasing size of the anterior part of the brain. Levi notes that this part of the skull has made more rapid development than any other in the interval between the 17 mm. and 28 mm. stages, and in the latter it is evidently still making rapid progress. When my model is compared with that of Hertwig, however, it is seen that in the period

between the 40 and 80 mm. stages the posterior fossa has made greater strides than the anterior, thus seeking to accommodate the enlarging, backwardly-growing cerebral hemispheres. The extent of development of the posterior portion of the brain-case in the interval between the 40 and 80 mm. stages will be realized when the ratio of the areas dorsal and ventral to the hypophyseal fossa, as they are found in the model of Hertwig and in my preparation, are compared. Although the dorsal area exceeds the ventral in my model the excess is by no means so great as it is in Hertwig's.

Speaking generally, since the 28 mm. stage of Levi there has been a flattening of the entire cranial floor. Between the 40 and 80 mm. stages the zone of greatest enlargement has been the upper edge of the posterior cranial fossa, the effect being as though this part had become stretched while the part around the foramen magnum had remained relatively stationary. The result is that the brain-case in this region has become more shallow and the sides more flaring, with their lateral and dorsal surfaces directed more caudally than outwardly. The region above the parietal plates has shared in this expansion, as is seen by the more widely placed parietal bones in the model of Hertwig, and this period of development has also witnessed the flattening out of the angle from the cranial aspect of the otic capsule, as may be seen when these skulls are compared.

Other expansive changes which have taken place in the interim between the 40 and 80 mm. stages are noted in the region of the middle ear, in the floor of the mouth, and in the temporal and zygomatic fossae, the details of which will be taken up in the discussion of the regions. In general one is struck with the large development of the cranial cavity, which gives to the Hertwig model a relative broadness when seen from the front, and has also resulted in a greater preponderance of the size of the cranium when compared with the facial region. A comparison of the Hertwig model with that of Jacoby for the 30 mm. stage brings out an even greater disproportion.

Of the skulls of other mammals those of *Macacus cynomolgus* and *Semnopithecus maurus*, modelled by Fischer ('03), may be

mentioned. The similarity in general outline is quite striking, when these are compared with my specimen. The illustration of the caudal aspect of the skull of *Macacus* shows a flattened condition of the partes cochleares closely analogous to that which obtains in my model. Though the dorsal part of the cranium is relatively shorter it is very suggestive of the cranium of homo, especially from the rudimentary condition of the side-walls.

CHONDROCRANIUM

Planum basale

That portion of the central stem of the chondrocranium which forms its dorso-caudal limb and is traversed medially and longitudinally by the notochord, is known as the planum basale (figs. 1, 2 and 5). It is an elongated and unperforated plate of cartilage of varying thickness, which extends from the intercondylar incisure of the foramen magnum to the dorsal border of the hypophyseal fossa, and forms the most dorsal part of the skull-base. Its cartilaginous substance is directly continuous with that of three regions; with the occipital region caudodorsally where the planum is seen to pass over into the lateral portions of the occipital anlage, with the orbitotemporal region cranio-ventrally, where it coalesces with the body of the sphenoidal cartilage at the dorsum sellae, and with the otic region laterally. The entire lateral border, with the exception of the extreme upper portion, is united to the pars cochlearis of the otic capsule, the line of union being indicated upon the medial surface of the latter by an elongated, narrow, crescentic strip (fig. 7). This line of union is formed of cartilage throughout, and in its cranial two-thirds this is of the same character as that of the adjoining parts. In the lower third, however, there is to be seen, microscopically, a distinct but thin sheet of younger cartilage, separating the adjacent parts of the planum and the cochlea, this being the last indication of the primitive separation of these parts.

It is worthy of note in passing that the 17 mm. stage of Levi shows the first indication of a union in the human skull between

the otic capsule and the planum basale, the cranialmost extremity of this bridge being the first to appear.

Encircling the zone of union of the pars cochlearis with the planum is a well-marked groove. The posterior portion of this, which may be known as the *dorsal basicochlear groove* (fig. 5), is crescentic in outline, and is fairly well defined. It contains the inferior petrosal sinus, and appears more sharply marked than in the models of Levi and Jacoby, judging from the illustrations of these authors. The ventral portion—much deeper and narrower—is also crescentic, and may be termed the *ventral basicochlear groove* (fig. 2). These grooves meet, above and below, their confluences being marked by notches, designated the *sphenocochlear* and *occipitocochlear notches* (fig. 5) respectively. The sphenocochlear notch occupies the interval between that part of the lateral surface of the corpus sphenoidale which lies dorsal to the processus alaris, medially, and the medial aspect of the cranial pole of the pars cochlearis, laterally (fig. 1). It is narrow and deep. The occipitocochlear notch is the ventromedial extremity of the jugular foramen.

Viewed from behind (figs. 1 and 5) the planum is seen to be concave from side to side in its lower two-thirds, and caudo-cranially throughout its entire extent. The cranial third of the dorsal surface terminates above in the crista transversa, a transverse ridge from which the dorsum sellae springs upwards, and which, according to Voit, may be taken to mark roughly the boundary between the body of the sphenoid and the otic or upper portion of the planum basale. This area of the dorsal surface is convex from side to side, and covers the part of the planum which has been called the clivus of Blumenbach. The lower portion of the planum is quite steep, but the cranial portion is much more so, the inclination thus agreeing with the description given by Levi for the 28 mm. stage.

The ventral surface of the planum (fig. 2) is convex from side to side, and almost straight caudo-cranially. It is thus evident that the caudal and cranial extremities are thicker than the middle portion, the latter being in the position of a primitive gap in the cartilage as shown by Levi in the 13 mm. and 14 mm.

stages and by Froriep in the 17.5 mm. stage, the latter having found the planum broken by a gap, filled with perichondrium, 1.9 mm. behind the canal of the hypophysis. This was just dorsal to the region where the occipitopharyngeal ligament, which had disappeared in older embryos (Levi), was inserted. This primitive gap (of which there is no evidence in my model, except the relative thinness of the cartilage in this region—and in this my findings agree with those of Levi and Jacoby for the 28 mm. and 30 mm. stages respectively—marks the site of the division which exists in early stages, between the two constituent parts of the planum, viz., that lying below it, belonging to the occipital region and representing the anlage of the future basi-occipitalis, and that lying above it, the clivus of Blumenbach, which is destined to form the basi-sphenoidalis, and has been included in the otic region.

The connection of the clivus with the body of the cartilaginous sphenoidal anlage is very primitive, according to the investigations of Levi upon young human embryos, as in the 13 mm. stage it is present while as yet there is no cartilaginous otic capsule in existence. This circumstance, combined with the relationship of the clivus to the basi-sphenoidalis of the osseous condition would seem to indicate that the upper portion of the planum should be grouped with the regio orbitotemporalis rather than with the regio otica, and this has been done by Levi, Jacoby and Van Noorden, but since Gaupp has more recently shown that the upper portion of the planum is more properly included with the otic region, and as several authors have since followed this course for the mammals (Voit, Mead) I shall adopt it in this description. The more minute details of the occipital and otic portions of the planum will be considered in the discussion of these regions.

The material composing the planum basale is mature cartilage, of uniform character, excepting one small, isolated mass of enlarged, vacuolated, cartilage cells, the nuclei of which appear larger and more darkly-staining than those of the surrounding areas, and the ground-substance of which has stained a dark

purple, thus standing in sharp contrast to the bluish matrix of the cartilage. This mass appears to be opposite the first part of the sub-basal course of the chorda dorsalis, and lies just ventro-caudal to the middle of the long axis of the planum. It belongs to the occipital portion, and is separated from the perichondrium by a thin sheet of mature cartilage. The perichondrium in the vicinity of this mass is unchanged, and contains no osseous tissue. In the 28 mm. stage of Levi the cells were, in this area, very large, and were surrounded by a very prominent capsule. We have in this area, no doubt, the beginning of the endochondral ossification center of the basi-occipitalis, which is said by Mall ('06) to appear on the 65th day.

The course of the chorda dorsalis has become familiar through numerous investigations, and I have nothing to add concerning it. The condition which I find agrees essentially with that illustrated by Fawcett for the 21 mm. stage (Fawcett '10a, fig. 2). The site of the future ligamentum suspensorium dentis, at the apex of the intercondyloid incisure, is marked by a coiled condition of the notochord, which corresponds to an intervertebral disk.

In my model the caudal portion of the planum is but slightly wider than the cranial, a condition which stands in sharp contrast to that found in the model of Hertwig, where one notices that, though the entire planum has undergone a widening, the region bordering the primitive foramen magnum has outstripped the more cranial portion in this regard. This broadening has, of course, separated the caudal extremities of the ventral and dorsal basicochlear grooves. The latter are both shallower and less strongly marked than in my model, as are also the sphenocochlear notches. Further, the side-to-side concavity in the model of Hertwig is almost obliterated, as is also that from above downward, and the inclination of the planum in the latter model is less steep than in mine.

Regio occipitalis

The occipital region has, in general, the form of a ring, whose irregular circumference, stoutly built and steeply sloping ventrally, plate-like and gently shelving dorsally, encloses the lower part of the posterior cranial fossa, the floor of which is perforated by an elongated fissure, the primitive foramen magnum (fig. 1). Its appearance suggests the occipital bone, of which it is the cartilaginous precursor.

Ventrally the ring is completed by a dorsally concave plate of cartilage, the basilar portion of the occipital anlage, which has been described as the lowermost part of the planum basale. As we have seen, the basilar portion is directly continuous cranially and laterally with the regio otica; below it splits to form the condyloid or lateral portions of the regio occipitalis, which spring downwards, outwards and slightly backwards and enclose, with their deviating, flattened limbs, the incisura intercondyloidea, marking the ventralmost part of the primitive foramen magnum. Reaching the most caudal points of the primitive skull at the paired, downwardly projecting ventral foramina prominences (figs. 2 and 3), upon the site of the future condyles of the occipital bone, they suddenly bend upwards and outwards, twist on their long axes so that the inner surfaces, which before looked dorso-medially now look principally cranially, and at the same time they broaden ventrally and laterally, their outermost borders coming to underlie and support the partes canaliculares of the otic capsules. The lateral wing-like plate, which is thus formed on each side, is really the ventral and narrowest part of the squamous portion, and is known as the lamina alaris (Voit) (fig. 5). Ventrally it terminates in the prominent processus paracondyloideus, (figs. 2, 3 and 4) which may be seen from the front projecting laterally from the outer surface of the condyloid portion; dorsally it broadens into the squama, which becomes steeper, and swings medially to pass into the tectum posterius (fig. 5). The upper border, after skirting the dorsal surface of the ear capsule, passes backwards and inwards, being continuous above

with the lower border of the parietal plate; the lower border forms the lateral and dorsal boundary of the primitive foramen magnum.

When regarded from the front (fig. 2) the lateral or condyloid portions appear as paired, caudo-lateral extensions of the planum basale, their outer surfaces being simply continuations of the ventral convex surface of the planum; or, stating the same thing another way, if the anterior surface of the planum be regarded as a section of a cylinder, then the outer surfaces of the lateral portions may be looked upon as localized widenings of the same. The upper boundary of each may be arbitrarily marked off by a line drawn from the tip of the occipitocochlear notch to the ventral foraminal prominence (fig. 5), cutting just ventral to the hypoglossal canal, and representing approximately the line of separation which exists between these elements as they occur in their osseous condition at birth. The caudal portion of the planum thus includes the intercondylar incisure. Piercing the outer surface of the lateral portion, which looks ventro-laterally, is seen the outlet of the hypoglossal canal (fig. 2). Upon reaching the external edge of the latter the outer surface becomes narrow, and passes directly outward upon the aforementioned processus paracondyloideus.

Seen from behind, the inner surfaces appear as caudolateral continuations of the side-to-side concavity of the dorsal surface of the planum (figs. 1 and 5). They look medially, dorsally and somewhat cranially, and present the inlet of the hypoglossal canal. The lower borders are by far the thicker, and form the lateral limits of the incisura intercondyloidea, and each, as has been noted, passes over the ventral foraminal prominence to be thence continued dorsally as the lateral border of the foramen magnum (fig. 2). This portion of the border of the foramen, and the lower border of the condyloid portion, much resemble one another in thickness and roundness on cross-section, and when the skull is regarded from the side the ventral foraminal prominence, formed by their approximation, appears as the apex of an angle directed downwards and slightly forwards (fig. 3).

The rounded upper border broadens dorso-laterally and bifurcates to enclose the hollow jugular recess (fig. 5), the ventral limb passing laterally to become the ventral border of the processus paracondyloideus (figs 2. and 4), while the dorsal is marked, at its termination, by a small eminence, the anlage of the future jugular tubercle (fig. 5).

The hypoglossal canal, whose inlet and outlet have been noted, pierces the condyloid portion in a direction from within outwards and forwards. It lies rather nearer the upper than the lower border, between the jugular tubercle above and the ventral foraminal prominence below. The right canal is unpartitioned, but the left presents a bar of cartilage which separates its inner third into cranio-ventral and caudo-dorsal inlets. This bar has a general direction from above downwards, forwards and inwards (fig. 5). The outer two-thirds of this canal is not divided.

When the sections are followed from behind forward it is noted that two fasciculi of the hypoglossal nerve come into close apposition, one with the other (though they remain, for a time, separated by their sheaths), just dorsal to the entrance of the hypoglossal canal. These are of about equal size, and pierce the dura as a single strand, to enter the canal (on the left side the caudo-dorsal inlet) after a short sub-dural course. A third strand, equal in size to the first two combined, may be seen to pierce the dura shortly after the first two, but remains separated from the latter (on the left side by the aforementioned septum) while traversing the canal. Upon emerging the strands unite and shortly after their exit they become intimately associated with the vagus. In the canal they are accompanied by some small veins—the anlage of the rete of the hypoglossal canal—and a small artery. The great bulk of the canal space is, however, filled with loose connective tissue.

The processus paracondyloideus,¹ already referred to more than once, forms a conspicuous object as it springs from the

¹ It may be here noted that Voit uses the term "processus paracondyloideus" to apply only to the outer projecting tip (as it is found in the skull of the rabbit) of the structure which I have designated by this term. Mead, in describing the skull of the pig, uses the name "processus paroccipitalis" with the same meaning

outer surface of the condyloid portion just lateral to the hypoglossal canal (figs. 2 and 4). The straight line which joins the outermost tips of the processes passes through their roots also, and meets the median sagittal plane at a right angle, thus showing that each process is perpendicular to the sagittal plane of the head. The coronal plane in which this line lies cuts the ventral foraminal prominences. Each process is prismatic in shape, and thus presents three surfaces, which meet at the most lateral point, or tip. The medial part of the cranial surface is hollowed for the recessus jugularis; the lateral part lies outside of the cranium (fig. 4), its convex area forming the outermost termination of the caudal delimitation of the jugular foramen. Immediately above this convex surface appears the proximal, curved end of the cartilage of Reichert. The remaining surfaces look ventro-caudally and dorso-caudally respectively, and are separated by the caudal border, which projects downwards in a ridge-like manner (figs. 2 and 3), and forms a prominent object, when the skull is regarded from below, as it springs laterally from the outer part of the ventral foraminal prominence. The dorsal border is continuous with the lamina alaris. The ventral border is free (figs. 2, 4 and 5), and is thin in its medial half, where it bounds the recessus jugularis ventrally. From within outwards it follows a curved line, convex ventrally, and, in the region of the recessus jugularis, there is a small cranial concavity, over which the jugular vein and accompanying nerves pass. As has been observed before, this ventral border is the anterior extension of the bifurcated upper border of the condyloid portion.

The left process presents a slight difference when compared with the right. A small foramen (figs. 2 and 5) is seen to tunnel under its ventral border, thus forming a passageway from the recessus jugularis within to the ventro-caudal surface of the process on the outer aspect of the skull. The outlet lies just lateral to that of the hypoglossal canal. Though the right side

as Voit gives to the term "processus paracondyloideus." I have selected the latter term, and used it in a more extended sense, as applying to the entire structure corresponding to the transverse (and perhaps costal) process of the occipital vertebra, since this represents a morphological unit.

does not present this foramen the cartilage in this locality is very thin. The foramen contains nothing but loose connective tissue and its direction is from within downwards and forwards. It may be known as the *paracondyloid foramen*, and appears in the model of Hertwig on the right side only.

The squamous portions form the dorsal, and most of the lateral, part of the occipital ring. The architectural, and, as we shall later see, possibly the developmental foundation of each half, is the crescentic bar of cartilage which forms the lateral boundary of the primitive foramen magnum, and extends between its two prominences (figs. 1, 2 and 5). Rounded in cross-section it is seen to diminish in size gradually and uniformly from before backwards. Ventrally it is directly continuous with the condyloid portion (and may even be looked upon as a backward extension of this), the area of union being marked by the anlage of the future condyle, which has been termed the ventral foraminal prominence. Its principal direction is dorsal and slightly cranial, in contrast to that of the condylar portion, which, as has been noted, is caudal, lateral and slightly dorsal. Its concavity looks medially and slightly caudally, the latter curvature being evident when the skull is regarded from the side (fig. 3). Dorsally it terminates in the paired dorsal foraminal prominences, which mark the entrances into the incisura occipitalis superior. As will be seen later this bar corresponds to the neural arch of the occipital vertebra, and will be hereafter referred to as such. Medial to it is the anlage of the future medulla oblongata. Just above its ventral portion appears the jugular tubercle, and, upon examining the cartilage in this location, the cells are seen to present, from the dorsal part of this tubercle to a point about midway between the foraminal prominences, a condition similar to that which obtains in the central part of the basilar portion. This would seem to point to the beginning endochondral ossification of the ex-occipital portion of the occipital bone, the center for which, according to Mall ('06) appears on the 56th day. The ventral part of this center is confined to the jugular tubercle, but, as the sections are followed backward, it is found that it gradually comes to involve the entire core of the neural arch. The involve-

ment in ossification of the jugular tubercle is to be noted, as this, as we shall later see, is probably to be regarded as the superior articular process of the occipital vertebra.

Springing laterally and dorsally from this neural arch we find the upward-shelving squama, which narrows ventrally into the lamina alaris, and dorso-medially participates in the formation of the tectum posterius. Its lateral portion is widened. The upper border of the squama may be divided into ventral and dorsal portions, the former being connected with the otic capsule, and the latter with the parietal plate (figs. 3 and 5). The ventral part is fitted closely to the caudal and dorsal surfaces of the pars canalicularis of the otic capsule, the line of union being crescentic in shape, with concavity looking upward, forward and outward. This border extends cranio-dorsally from the outer angle of the jugular foramen to the fissura capsulooccipitalis (Voit), and its position is marked on both the inner and outer aspects of the skull by crescentic grooves, formed by the approximation of the flattened occipital and rising otic surfaces. These furrows, which may be known as the *medial* and *lateral capsulo-occipital grooves* (figs. 3 and 5), are not equally well marked, that on the inside of the skull being much the deeper. It contains part of the transverse sinus.

Evidence of an earlier separation between the pars canalicularis and the squama is afforded by the microscopic appearance. Between these structures there is seen, ventrally, a thin sheet of perichondrium, its plane being parallel with the transverse planes of the head, and when the sections are followed dorsally this is found to give place to a cartilage of younger type than that surrounding it, this being traceable almost as far back as the capsulooccipital fissure. The younger condition of the intervening tissue in the region of the jugular foramen as compared with that farther back would seem to indicate that fusion of the parts has taken place in the more dorsal part first, and has gradually progressed forward, and this assumption is born out by examining the illustrations of Levi. In his 14 mm. model (which is the earliest stage in which the otic capsule appears) the pars canalicularis and squama are almost entirely separated

by an elongated fissure extending from the jugular foramen to a small bridge of cartilage which cuts off the capsulo occipital fissure, and it is believed that this long cleft represents the metotic fissure of the lower forms. Upon examining the illustration of Levi's 17 mm. stage it is found that the aforementioned small bridge of cartilage has considerably widened ventro-medially, and in the subsequent 28 mm. stage the squama and the pars canalicularis are united as far as the jugular foramen. In both the 17 and the 28 mm. stages of Levi the union was marked by a separating sheet of perichondrium.

At the caudo-ventral extremity of this union there is seen a small notch, passing laterally from the outer part of the jugular foramen to lose itself upon the external surface of the skull, just above and behind the tip of the paracondyloid process.

It may be noted that the otic capsule, between its dorso-lateral connection with the squama and its ventro-medial connection with the planum basale, forms a bridge, uniting these structures, roofing the recessus supraalaris, and affording an upper delimitation for the foramen jugulare.

Dorsal to the capsulooccipital fissure the upper border of the squama proceeds backwards and inwards, and describes a curve with concavity upwards, to reach a small eminence, seen in the Hertwig and other models, which may be known as the *dorsal occipital prominence* (figs. 1 and 2). Beyond this it falls away to join with the upper border of its partner of the opposite side, this junction resulting in the formation of a dorsal concavity, directed upwards, which marks the upper edge of the tectum. Between the capsulooccipital fissure and the dorsal occipital prominence the squama is continuous cranially with the parietal plate. The line of union of the two lies at the bottom of a groove, seen from the inner aspect of the skull. It may be known as the *occipitoparietal groove* (fig. 1) and presents, on the right side two perforations, on the left one, through which small veins pass. The paired foramen (known as the *occipitoparietal fissure*, (figs. 3 and 5), is the larger, is elongated, and is situated about midway between the extremities of the groove. It perforates the groove at the most caudal part of its course. The smaller

foramen, which is limited to the right side, appears just in front of the larger. On the right side the terminal fourth of the upper border of the squama is separated from the slender dorsal tip of the parietal plate by a narrow slit. The parietal plate appears to end freely, but dorsal to this there are what appear to be degenerating cartilaginous cells, connecting the end of the parietal plate with the squama. This may indicate that these structures were united at an earlier time.

I regret that the dorsal extremity of the head of my embryo is missing, and that I am, on that account, unable to ascertain the condition in this region. On the right side the sections terminate in the dorsal occipital prominence, and show that the parietal plate has come to an end before this, as described. Owing to the fact, however, that the sections were cut obliquely, being deeper on the right side than on the left, I am unable to say whether or not the termination of the left parietal plate, and the relations which it bears to the squama, are the same as those found on the right side. I have, however, assumed that they are, and have so constructed my model; this having been done there was only the gap between the dorsal occipital prominences to be filled in, and this I did by reference to the Levi illustrations and Hertwig model. There are indications, on the left side, that the separation of the posterior extremity of the parietal plate will take place, as it has on the right, the cartilage connecting it to the squama, in the last few sections, being very thin.

In the membrane just lateral to the tip of the right parietal plate and dorsal occipital prominence there appears the weakly staining spicule of the interparietal bone. I have not represented it in the model, since only a small fragment is available, the remainder being included in the missing sections.

The sections go back sufficiently far to show that the occipital squamae unite dorsal to the tip of the superior occipital incisure; to form the tectum posterius, thus differing from the findings of Voit ('09) in *lepus*, who states that the squamae never reach the midline, the edges of the superior occipital incisure being the dorsal borders. I am, of course, unable to ascertain the exact width of the tectum.

The ventral part of the lower border has been described as the neural arch. Dorsal to this the character of the border changes completely, for, after the dorsal foraminal prominence is passed it loses its thickness and roundness on section, and becomes thin and serrated. This portion borders the superior occipital incisure, and really represents the lower portion of the original dorsal border, the upper portion being united to form the tectum posterius. The latter, according to Levi's investigations, is formed somewhere between the 14 and 17 mm. stages by dorsal fusion of the squamae.

The surface outlined by the above-described boundaries has been seen to become narrower ventrally in the lamina alaris (fig. 5). This is a wing-like plate of cartilage, bounded ventrally by the processus paracondyloideus. The outer edge of its upper surface bears the caudal extremity of the pars canalicularis of the otic capsule, and medial to this the upper surface, which looks principally cranially, participates ventrally in the structure of the hollow recessus jugularis, and forms the floor of the recessus supraalaris. The latter is a cleft between the lamina and the overhanging pars canalicularis of the otic capsule (fig. 5) through the lateral part of which the transverse sinus passes. The lower surface of the lamina alaris is also hollowed in the more central parts of the paraforaminal area (fig. 2, p. 372), and the plate is, therefore, quite thin—in fact on the right side it is perforated by a minute foramen, just lateral to the neural arch (fig. 1), and posterior to the jugular tubercle, through which a small vein passes. This foramen is doubtless the representative of the condylar foramen of the adult condition. Dorsally the lamina alaris becomes wider, thicker, and more vertical in slope, as it passes into the larger posterior portion of the squama. The inner surface is smooth, and presents, dorso-lateral to the tip of the superior occipital incisure a small oval foramen on the right side, but not on the left, through which a small vein passes (fig. 1). A very shallow groove runs parallel to the neural arch, just lateral to its upper aspect.

Upon the outer surface the most prominent object is a rounded eminence, —the *lateral occipital eminence*—(fig. 3) which appears

immediately dorsal to the otic capsule, and is separated therefrom by the cranial portion of the lateral capsulooccipital groove. Its posterior part projects slightly into the cranial cavity (fig. 5), and its edges are ill-defined, fading gradually into the surrounding cartilage. This marks the thickest part of the squama. It may be followed caudally as a low ridge on the outer surface, and is seen to meet, almost at a right angle, a second ridge, which extends from the tip of the paracondyloid process to the dorsal foraminal prominence, and to which the name *crenescentic ridge* (figs. 2-3) may be given, the point of union being about the center of the latter.

The crescentic ridge, seen only on the outer surface of the skull, is a low elevation which sweeps dorsally and medially, between the extremities above mentioned, and in so doing describes a curve with convexity backwards and outwards. At its ventral end it is sharply defined, and is separated from the ear capsule by the small notch which stretches outward from the jugular foramen. Dorsally it is wider, and its margins are not so clearly outlined. It is of considerable thickness throughout, and is the lateral boundary of a semi-crescentic area—well seen in E. Fischer's ('03) illustration of the skull of *macacus cynomolgus*, and also in Voit's picture of the skull of *lepus*—which may be known as the *paraforaminal area* (fig. 2). It is bounded medially by the rounded edge of the neural arch, and ventrally by the caudal border of the paracondyloid process. Its hollowed central portion, which represents the lower surface of the lamina alaris, has been before referred to, and on the right side has been seen to be perforated by the minute condylar foramen. We find, therefore, that the thickest parts of the squama are situated immediately behind and below the ear capsule, and are represented by the lateral occipital eminence and the crescentic ridge, the former, just behind the capsulooccipital fissura, being slightly the thicker. The area dorsal to a line passing from the dorsal foraminal prominence to the occipitoparietal fissure is very thin, and presents a short distance behind this line evidence of commencing ossification. The cartilage appears to be undergoing greatest change in the region immediately dorso-lateral to the tip of the superior

occipital incisure, and dorsally the modified cartilage may be traced as far as my sections go. This area no doubt represents the center of ossification of the supraoccipital, which according to Mall ('06) ossifies from four centers, the first pair appearing in the region immediately dorsal to the foramen magnum on the 55th to 56th day, and being followed by other paired centers in the region ventral to this.

In all there have been noted in the occipital region five commencing centers of ossification, one, median, for the basilar portion; two, lateral for the condyloid portions, and two, lateral, for the squamous portions. In the vicinity of these centers the cartilage grades off into that surrounding them. Of the rest of the cartilage it may be noted that the material of the ventral border of the paracondyloid process, of the ventral part of the jugular tubercle, and of the body of the condyloid portion lateral to the hypoglossal canal shows a more advanced character than the remainder.

The atlantooccipital capsules are present; each is a sac composed of a dense sheet of perichondrium, covering the applied facets of the atlas and condyloid portion of the occipital cartilage. They are richly cellular, and above them the cartilage is of a younger type than that found in the remainder of the mass, but gradually grades off into the more mature type.

The primitive foramen magnum (figs. 1-2) is, at this stage, worthy of examination. It consists of three parts; a large central area, with which are continuous ventral and dorsal incisures. The central portion is bordered by the crescentic, rounded neural arch, and its plane looks upward and slightly forward. Ventrally is to be seen the intercondyloid incisure, its plane looking dorso-cranially, and this plane forms an angle with the main portion, which is open cranio-dorsally. This incisure remains a part of the foramen, later undergoing widening, and rounding out at the tip, as may be seen by examining the Hertwig model and the osseous skull. Behind the central portion, and appearing as a dorsal prolongation of it, is the superior occipital incisure, filled by the membrana atlanto-occipitalis dorsalis. The plane of this incisure is somewhat

steeper than that of the part in front, and thus it forms an angle, looking cranially and slightly ventrally, with that of the main area. This is shown upon the lateral contour of the skull as the point of an angle, directed downward and backward, marking the tip of the dorsal foraminal prominence (fig. 3).

The superior occipital incisure, though representing the dorsal part of the primitive foramen magnum, is by no means a part of the adult foramen magnum, as its edges unite later to complete the caudo-dorsal closure of the occipital region, the dorsal limit of the foramen magnum becoming set by the approximation of the dorsal foraminal prominences, as shown by the researches of Bolk ('04).

I find no evidences of the condition which Bolk describes at the posterior extremity of the foramen magnum, viz., a central cartilaginous mass (formed by the fusion of paired pre-existing masses) which lies between the upturned dorsal extremities of the occipital side-walls.

It seems clear from the work of various investigators, beginning with Froriep ('86), that although the occipital region of the mammals has been developed from the skeletogenous elements of four metameres, only the most caudal ever attains the status of a mature sclerotome, the three cranialmost being undifferentiated and playing but a minor part in the construction of the adult bone. This being true it follows that the story of the evolution of the occipital anlage is largely the story of the development of the caudal segment, or, as it will be hereafter called, the occipital scleromere or primitive occipital vertebra.

As in the spinal region, so in the occipital, the sclerogenous tissue passes through successive and overlapping membranous or blastemal, chondrogenous and osteogenous phases (Bardeen '05, '08), and in each phase the condition in the occiput recalls that of the corresponding phase in the vertebrae. Thus in the blastemal stage the occipital scleromere shows paired chordal processes joining across the midline in the region of the notochord, and paired neural processes embracing the neural canal as in the spinal scleromere; the costal processes are, apparently, poorly developed in the occipital scleromere. But along with this

marked similarity to the vertebrae the occipital scleromere shows certain individual peculiarities. Instead of uniting with the cranial portion of the segment immediately caudal to it, after the fashion of the other scleromeres, it retains its connection with the cranial portion of its own segment, and this, in turn, becomes united with the tissue of the three cranial, undifferentiated sclerotomes, the membranous anlage thus formed being known as the occipital plate (Bardeen '08 and '10). According to Frioriep the occipital scleromere is marked off from the undifferentiated sclerotomes by the caudal root of the hypoglossal nerve.

The middlepiece of the occipital plate is made up in its caudal portion of the chordal processes of the occipital scleromere, and in its cranial part it also contains the elements of the body masses of the undifferentiated segments. So also while the lateral portions are mainly formed of the neural processes of the occipital scleromere they also contain, in the region cranial to the hypoglossal canal, remnants of the condensed lateral masses of the undifferentiated segments (Frioriep, Levi).

In the chondrogenous stage of the occipital anlage of man, for the knowledge of which we are principally indebted to Levi, there are also striking resemblances to the vertebral conditions. The 13 mm. stage, studied by this author, shows the beginning of the transition from the membranous to the cartilaginous condition and in this paired masses of condensed chondrogenic mesenchyme, separated by the perichordal septum, were situated dorso-caudally in the middlepiece or basilar portion, and in the dorsal part of each mass, medial to the hypoglossal nerve, a small cartilaginous nodule occurred, recalling the paired chondrous centers of the body of a spinal vertebra. The mesenchymatous masses, representing the chordal processes of the occipital scleromere, were joined ventrally; they are the first portions of the occipital anlage, and indeed of the entire chondrocranium, to undergo chondrification, and eventually form the diverging, caudolateral portions of the pars basilaris bordering the foramen magnum. In the matter of priority in time of chondrification

of the body over the arch processes the occipital scleromere resembles those of the atlas and axis.

In the 14 mm. stage of Levi chondrous paired centers for the neural processes of the occipital vertebra arise, lateral to the hypoglossal nerve roots, and speedily unite by continuity of cartilage with the nodules just mentioned, which also join with one another in the same way, at first ventrally. The fused basilar centers are a little later joined by the chondrifying middle part of the undifferentiated sclerotomes to form the median part of the pars basilaris, and the chondrifying lateral masses of the undifferentiated sclerotomes join the neural processes. Thus the basilar portion, which may be taken to extend to the ventral margin of the hypoglossal canal is built up from the middle parts of all of the primitive occipital segments, the portion included in the anterior margin of the foramen magnum arising from the body mass of the occipital scleromere, and the cranioventral part coming from the body masses of the undifferentiated sclerotomes.

A lateral outgrowth from the neural arch is seen in a membranous condition in the 13 mm. stage of Levi, and is somewhat later in chondrifying than the neural arch of either the occipital vertebra or of the atlas. It represents, doubtless, the transverse process of the occipital vertebra, and is spoken of by Levi as the Querleiste. The costal process of the occipital vertebra has not been shown to have a separate center of chondrification.

It is to be noted that in the 13 mm. stage of Levi the bodies of the occipital vertebra, atlas and axis are represented by paired masses of chondrogenic mesenchyme, separated by the perichordal septum, and each mass contains a small nodule of cartilage (except in the case of the axis, where the two nodules have fused), their neural arch processes being entirely membranous. In the 14 mm. stage of Levi not only are the bodies chondrified but also the neural arches; for in the occipital vertebra a chondrous center appears just lateral to the hypoglossal foramen, and the arches of the atlas and axis also present each a small nodule of cartilage. Thus chondrification takes place simultaneously in cor-

responding parts of the occipital and first and second spinal vertebrae.

The paired cartilages of the body of the occipital scleromere of Levi's 14 mm. stage appear to be slow in joining dorsally, being found separated in this region by the perichordal septum, while their ventral parts are united. This is possibly to be explained as a result of expansion in this region, from intracranial pressure.

The above identification of the cartilaginous neural arch of the occipital vertebra and its appendage, the transverse process, as found in the early Levi models does not agree with this author's own interpretations of his findings. In his earliest human skull, from a 13 mm. embryo, Levi shows, lateral to the roots of the hypoglossal nerve, what he calls the lateral portions, from whose lateral surfaces the Querleisten project directly outward, and he figures the latter in all four of his stages. In the 14 mm. stage he finds in each lateral portion, just external to the hypoglossal nerve roots (Levi, text fig. 2) a cartilaginous center, which speedily joins with that for the body mass, which latter, as has already been noted, represents the chondrification of the body of the occipital scleromere. It seems evident that the center of chondrification in Levi's lateral portion is the center for the neural process of the occipital scleromere, with possibly the addition of the center for the undifferentiated portion, and hence it follows that the club-like membranous mass in which this nodule is found is the neural process of the occipital scleromere, which, with its partner, builds the lateral part of the neural canal of the occiput. Furthermore, these lateral portions are in direct alignment with the spreading arch-processes of the underlying cervical vertebrae, as is shown by the illustrations of Levi, and, though this author does not label them as the arches of the occipital vertebra, Bardeen, in his copy of Levi's illustration of his 13 mm. stage in Keibel and Mall's "Human Embryology" (vol. 1, p. 401) gives them what I regard as the correct designation, "Arcus vert. occip." Though the lateral portions of the occipital vertebra are here considerably larger than the arches of a cervical vertebra this extra size is probably

a local adaptation. Again, at the same time (14 mm. stage) according to Levi the arch mass of the undifferentiated sclerotomes has chondrified. It would, indeed, be surprising if the neural arch of the occipital vertebra were still membranous at a time when the arch processes of the undifferentiated sclerotomes were chondrified (those of the atlas and axis being also chondrified), as would be the case if Levi's interpretation were correct. My identification obviates this difficulty.

Accepting this interpretation it follows that the small processes or Querleisten which project laterally from the lateral processes cannot be the tips of the neural arches, as Levi describes them; they are really the anlagen of the transverse processes of the occipital vertebra. Levi, who does not account for the dorsal tips of the lateral portions of the occipital vertebra at all, is led to conclude that the Querleisten represent the tips of the neural arches, apparently, by the histological resemblance of their tissue, in the early stages, to that composing the tips of the neural arches of the underlying cervical vertebra. He remarks, however, that the Querleiste is very tardy in chondrifying when compared with the neural tip of the atlas, a detail which is, if anything, opposed to his identification of it as the tip of the neural arch of the occipital vertebra, but is what might be expected if it be homologized with a transverse process. The Querleisten are shown in the Levi models to be in direct alignment with the transverse processes of the cervical vertebrae, and they never come together dorsally and unite, after the fashion of the dorsal extremities of the neural arches. They are identical with what I have called in my model the paracondyloid process, following Voit, who described similar structures in the rabbit, and identified them as the representatives of the transverse processes, also remarking that the rectus capitis lateralis muscle, which each has attached to its lower surface, is to be regarded as the morphological equivalent of an intertransversarius muscle. Mead ('09), too, finds a similar and very strongly marked process in the skull of the pig, and calls it the paroccipital process. Both Levi and Voit state, correctly I believe, that the process ultimately becomes the jugular process of the occipital bone.

My identification of the primitive elements forming the pars basilaris also is not exactly in agreement with Levi's, but the difference depends largely on where the line between the basilar and lateral portions of the occipital anlage is drawn. I have assumed the separation between these portions to be approximately as it exists at birth, while Levi includes in his lateral portions the nodules which I believe represent the paired body of the occipital vertebra. If my interpretation of these structures is correct they should be regarded as constituents of the basilar portion, which would thus represent the body masses of the undifferentiated sclerotomes plus the body mass of the occipital vertebra, while according to Levi's view it would represent only the body masses of the undifferentiated vertebrae.

In the later development of the chondrogenous stage the forerunners of the individual features of the occipital bone begin to show themselves, and we find cartilaginous representatives of the body, pedicles, inferior and superior articular processes, transverse (and possibly costal) processes, laminae and spinous processes, these almost altogether differentiating from the occipital scleromere. The development of the body (pars basilaris) has already been discussed. The pedicles are, of course, represented by the cartilaginous tissue in the region of the hypoglossal foramen, but here we have material added from the lateral mass of the cranial sclerotomes, (Froriep, Levi), which results sometimes in the partitioning of the foramen, as may be seen on the left side of my model, and as has frequently been found by other observers in young embryos of homo and other mammals. The inferior articular process is, doubtless, represented by what has been designated the ventral foraminal prominence, the forerunner of the condyle, though the condyle of the mature bone is partly formed by the pars basilaris. The rudiment of the superior articular process is, perhaps, to be seen in the jugular tubercle. The transverse process, as we have seen, is to be found in the paracondyloid process, and there is some evidence to indicate that in this latter there may be included the costal process as well. It will be remembered that the paracondyloid process was perforated on the left side by

the paracondyloid foramen, and that on the right side the corresponding area of cartilage was thin, and, further, that the Hertwig model also presented this foramen on one side. It pierces the process from above downwards and forwards and is in series with the costo-transverse foramina of the cervical vertebrae below. If it be regarded as the costo-transverse foramen of the occipital vertebra, then the bar of cartilage which closes it in front must be looked upon as the costal process of this vertebra. I have not been able to find that the costal process of the occipital anlage has a separate center of chondrification as it has in the spinal vertebrae. Though there is no vessel or nerve passing through the paracondyloid foramen yet it is possible that this represents an old channel of the vertebral artery, which has become obsolete on account of the change of course of this vessel. In this regard the foramen may be analogous to the costo-transverse foramen of the 7th cervical vertebra, which no longer transmits the vertebral artery.

I regard as the representatives of the laminae the crescentic, tapering, hornlike masses of cartilage which form the lateral borders of the foramen magnum, and which I have spoken of as the neural arches. Since the squamous portions are continuous with the outer borders of these they may be considered as extensions of the laminae, and the tips of the latter (dorsal foraminal prominences) as the representatives of the spinous processes.

Not only on developmental, but also on histological grounds, does it appear evident that the margin of the foramen magnum is formed from the primitive neural arch of the occipital vertebra. Upon an examination of my slides it is seen that the cartilaginous tissue of this portion bears a strong resemblance to that composing the arches of the upper cervical vertebrae, and even more striking is the situation of the ossification center. It appears, as I have described, in the arch of the occipital vertebra, just dorsal to the root of the transverse process—exactly the same relative position as the ossification center in a cervical vertebral arch occupies. This point is beautifully brought out in the model of Hertwig, where, upon either side of the neural canal a

series of ossification centers presents itself, the uppermost member of the series being found in the exoccipital, in the position in which I have described it in my model, and being followed caudally by the ossification centers for the 1st, 2nd, 3rd and 4th cervical vertebrae; each center being uniformly situated with reference to its respective arch. In the basi-occipital, too, the single median ossification center recalls the center of a typical vertebral body, and indeed the osseous elements of the occipital bone at birth are strikingly similar to those of a vertebra, especially the fifth lumbar vertebra.

Keeping in mind the intimate and peculiar relationship which a typical vertebral arch bears to the cord which it encloses it seems reasonable to suppose that this relationship would be retained even after the vertebra had been taken up into the skull. It seems much more reasonable, even on purely theoretical grounds, to assume that the arch of the occipital vertebra goes to form the margin of the foramen magnum of the mature skull than to postulate that it forms the jugular process, as Levi maintains, and when it is realized that the latter is formed from the transverse process of the occipital vertebra, and that the margin of the foramen magnum is merely the modified arch of the occipital vertebra the proper relationship of the parts becomes intelligible.

Not only do the neural arches of the occipital vertebra exhibit a striking resemblance to those of the cervical vertebrae in each stage which has been studied, but their behavior in growth recalls very strongly that of the arches below. This parallelism in manner and time of development between the arch of the occipital vertebra and those of the cervical vertebrae is clearly shown by an examination of the 13 mm., 14 mm., 17 mm., and 28 mm. stages of Levi, my own 40 mm. stage, and the 80 mm. stage of Hertwig. The neural arches of the occipital vertebra, small at first, are seen to grow backwards and outwards, and then to come together medially and dorsally, thus hedging in the dorsal part of the foramen magnum, this process being duplicated coincidentally by each of the upper cervical vertebral arches.

In each successive stage the tips of the neural arches, both occipital and cervical (which retain their original alignment), are seen to be farther advanced than in the last in their enclosure of the spinal cord, a condition strikingly brought out by a comparison of the foramen magnum and the underlying vertebral arches of my model with those of the oldest Levi model, on the one hand, and the Hertwig model on the other. In the 28 mm. Levi model the tips of the neural arches of the occipital and upper cervical vertebrae are separated by a considerable interval, in my model they are almost united, and in the Hertwig model, as has been noted, they are all completely joined; in the adult bone the tips of the occipital vertebra are represented by the internal and external occipital crests (representative of a spinous process). This closure of the foramen magnum takes place, accordingly, somewhere between the 40 and 80 mm. stages in man, and it bears a striking resemblance to that of the segments of the spinal canal. Growth seems to progress uniformly throughout the series, and dorsal closure is apparently completed at about the same time in each segment. Thus, with the fusion of the dorsal foraminal prominences there is completed what amounts to the closure of the cranial extremity of the spinal canal.

From what has been said regarding the formation of the foramen magnum it will be evident that what is found in the 40 mm. stage is something more than the foramen of the adult condition; it is this plus the superior occipital incisure. Further, the structure described as the tectum posterius is not the dorsal delimitation of the real foramen magnum at all, but merely that of the superior occipital incisure. The edges of the latter unite in the form of a median seam upon the union of the dorsal extremities of the neural arches of the occipital vertebra, and thus is effected the closure of the portion of the floor of the occipital region dorsal to the foramen magnum. This conception of the development of the foramen magnum explains why the primitive foramen is relatively so much larger than the adult condition.

In the condition of the occipital anlage at birth we find a basilar portion, formed in its cranial part from the body mass

of the undifferentiated sclerotomes and in its foraminal portion from the body mass of the occipital vertebra. The exoccipitals have been developed, as we have seen, principally from the neural arches of the occipital scleromere, but in the region of the foramen hypoglossi there has been added material from the lateral masses of the undifferentiated scleromere. The supraoccipitals, which ossify separately, are to be regarded as primarily connected with the neural arch of the occipital vertebra, and their separateness of ossification is analogous to the condition which we find in the 5th lumbar vertebra.

Regio otica

The otic region, like the occipital, is transversely somewhat ringlike in form, and its irregular sides, for the most part flattened from within outwards, are united by their caudal edges with the upper border of the occipital anlage, except where sundry foramina occasion interruptions. The otic ring, accordingly, heightens the dorsal part of the cartilaginous brain-case. In it we recognize four distinct elements, two unpaired, the otic portion of the lamina basalis and the tectum synoticum, and two paired, the otic capsule and the lamina parietalis. In addition to the parts entering into the composition of the ring there are also to be considered in the otic region the small, paired, isolated nodules known as the cartilaginee supracochleares, cranii laterales and cranii posteriores.

When the skull is viewed from within (figs. 1 and 5) the upper or otic portion of the lamina basalis is seen to unite the ventro-median portions of the otic capsules. Passing laterally the eye meets the large, irregular mass of cartilage known as the otic capsule, which forms the ventro-lateral delimitation of the posterior cranial fossa, as well as part of the floor of the middle cranial fossa. Caudo-laterally the capsule is continuous with the lamina alaris, and dorsal to this with the wider portion of the squama of the occipital anlage, while cranio-dorsally the commissura capsuloparietalis (figs. 3 and 5) is seen uniting the larger dorsal portion of the otic capsule with the lamina parie-

talis the latter having also a union with the capsule below the capsuloparietal fissure. Dorsal to the capsuloparietal commissure the flattened parietal plate appears, and we note that it is wide ventrally, but becomes narrow dorso-medially. With the parietal bone it assists in the formation of the wall of the cranium in this region. Below, the parietal plate is continuous with the upper border of the squama; dorsally it is represented in the model as terminating freely just before reaching the dorsal occipital prominence, but there is microscopical evidence, as far as my sections go (as I have already stated) which seems to indicate that there was here a previous union of parietal plate and squama. Of the unpaired, dorso-median tectum synoticum, described by several authors, I can, unfortunately, make no statement, as my sections for this dorsalmost region are lacking. The otic ring, as I have represented it in the model, is therefore incomplete dorsally. This may possibly be its actual condition, and in this connection it may be noted that Mead states that the otic ring in *Sus* is incomplete dorsally.

The pars otica of the lamina basalis, which is the most cranial part of the chordal portion of the base of the skull, has already been described. The fissura basicochlearis is incomplete above, being represented by the lowermost part of what I have designated the spheno-cochlear notch—filled with connective tissue and a few small veins. The abducens nerve passes above the notch, lateral to and below the outwardly-projecting posterior clinoid processes, and in this the condition is similar to that described in such mammals as the rabbit (Voit) and pig (Mead), except that the basi-cochlear fissure in the latter types is closed above by a cartilaginous bridge joining the upper surface of the pars cochlearis with the lamina basalis, the abducens nerve passing over this bridge. In the model of *Sus* by Mead this nerve passes through a foramen formed by cartilaginous connection of the posterior clinoid process with the cochlea. There is no evidence of this in my preparation.

The connections of the otic capsule with the planum basale, squama occipitalis and parietal plate have been noted. The model also shows it continuous ventro-laterally with the incus,

but histologically a sheet of perichondrium intervenes. There is no connection with the processus alaris of the temporal region through the commissura alicochlearis, such as Jacoby shows in the 30 mm. stage of homo and Voit figures in his model of the skull of lepus, but there is what I regard as a rudiment of this, viz., the cartilago supracochlearis, (figs. 1 and 3) which will be later described. The otic capsule roofs over the recessus supra-alaris and recessus jugularis, and bridges the foramen jugulare (fig. 5).

In the otic capsule (figs. 6 and 7) we may distinguish a larger dorsolateral portion, which contains the semicircular canals, and which may therefore be known as the pars canalicularis (Voit), and a smaller, ventro-median portion, which contains the cochlear part of the membranous labyrinth, and which may therefore be termed the pars cochlearis. Voit has restricted the use of the term 'pars vestibularis' to the dorsal part of the pars cochlearis, which presents the fenestrae vestibuli and perilymphatica and the fenestrae for the vestibular division of the eighth cranial nerve. It contains the first, or unwound, portion of the ductus cochlearis. I shall adopt this usage of the term in this description.

The cartilage of the two portions is directly continuous, the zone of union being marked cranially by a notch, open above, which may be known as the *superior otic notch* (figs. 6 and 7), and ventro-laterally by a recess formed by the union of the lateral surface of the pars cochlearis with the ventral surface of the pars canalicularis; this may be known as the *ventro-lateral otic recess* (fig. 6). It contains the anlagen of the auditory ossicles.

The pars canalicularis is an irregular, somewhat flattened, ovoid mass of cartilage, hollowed for the passageways of the semicircular canals and utriculus. It presents for examination three surfaces, ventral, lateral and medial. Of these the lateral and medial are convex, and are approximated above and behind, their ventral edges being widely separated. The lateral surface (fig. 6) is smooth, and somewhat triangular in shape, being wider above than below. The cranial border is rounded, and is formed

by the out-bulging of the anterior semicircular canal; it is known as the *prominentia semicircularis anterior*. Ventrally this prominence terminates in the *prominentia utriculo-ampullaris superior*, a conspicuous rounded eminence at the cranio-ventral extremity of the *pars canalicularis*, marking the upper approximation point of its three surfaces. It is formed principally by the wall of the ampulla of the anterior semicircular canal (fig. 8). Springing upward from the dorsal part of the anterior semicircular prominence the capsuloparietal commissure may be seen, its connection with the otic capsule being shown in figures 6 and 7. Dorsal to this the border is marked by the capsuloparietal fissure (fig. 3), and caudal to this again by the lower union of the parietal plate with the otic capsule; under the latter union is to be noted the capsulooccipital fissure (fig. 7). The lowermost part of this border is formed by union with the squama. These borders separate the lateral from the medial surface of the capsule.

Both the capsuloparietal and capsulooccipital fissures appear in other models of the human skull (Levi, Hertwig), and they have also been shown to be present in the primitive skulls of other mammals, as the ape (Fischer) and rabbit (Voit). The capsuloparietal fissure is sometimes known as the *foramen jugulare spurium*, and the capsulooccipital fissure as the *foramen petrosooccipitale*.

The ventral border, which separates the lateral from the ventral surface, is, below the superior utriculoampullary prominence, marked off mainly by the conspicuous *crista parotica*, below this by the mastoid process, and below this again by the *prominentia semicircularis posterior* (fig. 6), which passes over the root of the mastoid process at this point.

The most prominent object upon the lateral surface is the *lateral otic eminence* (fig. 6), which lies in its dorso-cranial area, separated from the dorsal part of the anterior semicircular prominence by a very shallow groove. It slopes backward into the parietal plate between the two post-otic fissures, and is formed by the backward and outward projection of the *massa angularis*, a large mass of cartilage lying in the enclosure formed by the anterior and lateral semicircular canals, the *crus com-*

mune, and the upper part of the posterior canal (fig. 8). The two lateral otic eminences mark the extremities of the greatest transverse diameter of the primitive skull.

The dorsal extremity of the posterior semicircular prominence forms a gentle rise in the caudal area of the lateral surface, and then passes over the root of the mastoid process, as we have seen, to become prominent in its ventralmost portion, the *prominentia utriculoampullaris inferior* (figs. 6 and 7), which forms the conspicuous border between the ventral and medial surfaces and acts as the upper border of the lateral part of the jugular foramen.

The *prominentia semicircularis lateralis* is an indistinct swelling passing downward and backward from the *crista parotica* in the region of the incus to the dorsal extremity of the posterior semicircular prominence.

The medial surface (fig. 7) is more extensive than the lateral. Its cranial and dorsal borders are the same as those of the lateral surface; its ventral border is marked above by a rounded ridge passing downwards from the superior utriculoampullary prominence to the superior otic notch, and below by the posterior semicircular prominence, which, as has been seen, terminates ventro-medially in the inferior utriculoampullary prominence, the latter bearing a ventrally-projecting process, the *processus interperilymphatica* (Voit). The middle portion of the ventral boundary is formed by the transition of the medial surface of the *pars canalicularis* into that of the vestibular portion of the *pars cochlearis*. As has been mentioned the medial surface is convex, and presents in its central area as its most prominent object the *prominentia cruris communis* (Voit), formed by the *crus commune* within (fig. 9). Upon the dorsal part of this prominence is seen the long, almost horizontal, slit-like foramen *endolymphaticum*, for the outlet of the *ductus endolymphaticus*. Both lips of this foramen are formed of a young type of cartilage and it may be noted that the upper lip projects medially in its dorsal part to overhang the duct, and is continued dorsally past the foramen to form a groove, in which the duct lies (fig. 7). The dorsal extremity of this upper lip appears as a short, free process, overlying the duct.

The condition of the terminal portion of the ductus endolymphaticus is of interest. This does not end in a sac, but becomes a long, narrow fusiform dilation shortly after emerging from the foramen, and gradually decreases in size, to be prolonged, at its dorsal extremity, into a fine, lumenless filament or cord of cells. After leaving the foramen endolymphaticum it passes medial to the transverse sinus in the sub-dural space, outward and backward, and ends in the loose sub-dural connective tissue just medial to the occipitoparietal groove, about 1.8 cm. dorsal to the capsuloparietal fissure. It is not intimately associated with the cartilage of the ear capsule after its exit therefrom, and hence cannot retard the development of this locally to bring about a thinness of the wall, which is found in my preparation just dorsal to the endolymphatic foramen, in the area corresponding to that in which the small foramen which Voit describes in the developing otic capsule of the rabbit appears. This thin region of the wall (which is unperforated) is caused by encroachment upon it from within of the cavities surrounding the dorsal extremities of the anterior and posterior semicircular canals, and not by pressure of the saccus endolymphaticus from without, as Voit assumes in the skull of lepus.

The upper part of the medial surface is marked by the crescentic inner aspect of the anterior semicircular prominence (fig. 7), which is more distinct here than on the lateral surface, and sweeps backwards, from the superior utriculoampullary prominence to the dorsal end of the prominence of the crus commune. It corresponds to the arcuate eminence of the adult bone. Below this prominence is to be seen a distinct fossa, the fossa subarcuata anterior (Voit), delimited caudally by the prominence of the crus commune. This fossa invades the substance of the massa angularis, and upon examining the slides microscopically it is found that it is filled with a mass of loose connective tissue, covered by the dura.

The medial surface below the prominence of the crus commune looks downward, backward and inward in its upper portion, and almost directly downward in the lower. The latter is thin, composed of more darkly staining cartilage with thickset cells and little ground substance, and forms the roof of the supraalar

recess. In its ventral area may be seen the inferior utriculo-ampullary prominence, continuous dorso-laterally with the posterior semicircular prominence and ventrally with the short interperilymphatic process. A spur of the inferior utriculo-ampullary prominence caused by a localized thickening of the wall, projects backwards and upwards as a low ridge to disappear somewhat below the endolymphatic foramen. It overhangs the transverse sinus in this region. The fossa subarcuata posterior, which Voit mentions in his description of the skull of the rabbit, is not represented here.

The boundaries of the ventral surface (fig. 6) have already been described in connection with the discussion of the ventral boundaries of the lateral and medial surfaces. Its medial part is concerned with the junction of the pars canalicularis with the vestibular portion of the pars cochlearis. The lateral part of the ventral surface forms the dorsal wall of the ventro-lateral otic recess, which has been already referred to, and which contains the structures entering into the formation of the middle ear.

In the cranial area of the ventral surface there appears, projecting forward from the ventral surface of the superior utriculoampullary prominence, a distinct, almost vertical, ridge (figs. 5 and 6), which lies immediately medial to the body of the malleus, but is separated therefrom by a sheet of connective tissue. This represents the medial part of the cartilaginous tegmen tympani, or processus perioticus superior. The lateral portion of the tegmen, such as is shown in Voit's model of the skull of *lepus*, is not present, but its position is indicated by a low ridge, which arches downward and outward from the upper end of the medial portion of the tegmen and marks off the ventral from the lateral surface, terminating below in the crista parotica (fig. 6). The cartilage is not developing rapidly in this location, as in Voit's specimen. Upon examining the model of Hertwig it is found that the tegmen tympani has grown forward and outward to overlie partially the bodies of the malleus and incus, but the lateral portion has evidently made no further development, and so it may be concluded that the ossicles in man do not occupy a deep cartilaginous recess formed by the teg-

men tympani as they do in the rabbit. In the oldest rabbit skull examined by Voit the lateral portion of the tegmen had become quite a prominent plate, covering the ossicles, and reaching out toward the lateral wall of the middle cranial fossa. It appears evident that the tegmen tympani is rudimentary in man.

The crista parotica (figs. 2, 3 and 6) forms a conspicuous object upon the border between the ventral and lateral surfaces. It is narrower, as well as more prominent, below than above, and its edge shows younger cartilage than the adjacent regions. The cartilage of the incus, though in the model it appears to be attached, is really quite separate from that of the ear capsule, there being an intervening sheet of perichondrium.

The lowermost part of the ventral surface lies in a somewhat more posterior plane than the upper, and forms the dorsal wall of a small recess, open in front and below, bounded laterally by the crista parotica and medially by the interfenestral septum of the vestibular portion of the *pars cochlearis* (or promontorium). In the upper and medial part of this recess appears the lower portion of the fenestra vestibuli, while in the lateral portion, sheltered by the lower part of the crista, the facial nerve is to be found, this region becoming later the lower part of the facial canal or aqueduct of Fallopius. The proximal end of the cartilage of Reichert may be seen just medial to the lower extremity of the crista (fig. 2).

Just below the crista, and separated from it by a small notch, there appears, on the right side, a short, free, anteriorly projecting conical spur of cartilage, slightly younger in character than that of the adjacent otic capsule, and representing the mastoid process of the adult condition (fig. 6). Its substance is directly continuous with that of the ear capsule dorsally, but medially it is separated therefrom by perichondrium. On the left side the same formation is to be seen, except that a portion of the intervening sheet of perichondrium is, near the point of the process, replaced by cartilage. Immediately medial to each process is to be seen the origin of the stapedius muscle.

A brief word as to the course of the facial nerve may here be in place. After entering the internal acoustic meatus it traverses

the facial foramen in a direction outward and slightly forward and enters the ventro-lateral otic recess. Here it becomes associated with the geniculate ganglion from which the great superficial petrosal nerve may be followed forward. Leaving the geniculate ganglion the facial nerve now passes downward and slightly outward over the large cartilaginous bar which unites the pars cochlearis with the pars canalicularis and which is found between the facial foramen above and the vestibular fenestra below; thence it proceeds backward over the incudo-stapedial articulation. It now is to be found just medial to the crista parotica, and runs steeply downward, the relatively small stapedial muscle lying medial to it here. Passing lateral to the upturned end of the cartilage of Reichert, just between the latter and the lower tip of the crista parotica, it gives off the chorda tympani, and turns suddenly forward, following the line of the shaft of Reichert's cartilage, being situated slightly above and lateral to it, and almost immediately lateral to the auditory or Eustachian tube. The relations of the facial nerve at the proximal end of Reichert's cartilage are those shown in Low's ('09) plate, figure 3. The chorda follows its wellknown course through the middle ear anlage.

It is to be noted that the facial nerve does not go through a secondary facial foramen formed by the connection of the tegmen tympani ventrally with the cochlea, as is the case in the rabbit (Voit), and hence there is no true fovea genicularis in the skull of man in this stage, or, indeed, in any stage, judging from the evidence at hand.

The slightly younger condition of the cartilage along the ventral margin of the crista parotica would seem to indicate that the facial canal was closing here, but in the Hertwig model it is still open at this region.

The walls of the pars canalicularis are for the most part thin, and composed of mature cartilage. The largest mass of cartilage is formed by the massa angularis, mentioned above, the ventro-median side of which lies immediately lateral to the fossa subarcuata anterior, while the dorso-lateral side projects outward as the lateral otic eminence. Within the mass, just below the

floor of the anterior subarcuate fossa, there is a small isolated cavity, and as this is within the arch of the anterior semicircular canal I regard it as a remnant of a portion of the otocyst, which is, as yet, unclosed here. The ground substance of the cartilage of this mass is abundant and pale staining, the nuclei being relatively scattered, and surrounded by capsules not increased in size.

The remainder of the cartilage of the pars canicularis is made up of masses filling the interstices between the canals and ampullae.

The pars cochlearis is the anterior and smaller part of the otic capsule, and lies immediately lateral to the upper end of the basal plate (figs. 1 and 2). Like the pars canicularis it is of flattened, ovoid form, and contains the sacculus and ductus cochlearis. Upon it we may recognize two principal surfaces, medial and lateral, to which may be added a third or caudal surface, made up of the structures in the vestibular portion surrounding the foramen perilymphaticum.

The medial and lateral surfaces are separated by a rounded border, which runs from the perilymphatic foramen below, around the ventral part of the pars cochlearis, over the cranial pole, and thence backward to terminate by passing over the suprafacial commissure to become continuous with the pars canicularis at the superior otic notch. The lowermost part of this border is deflected outward to form the promontory; within it is the first and uncoiled part of the cochlear duct, and it is known as the *prominentia cochlearis inferior* (Voit) (figs. 2, 6 and 7). This prominence passes at first inward, forward and upward, then almost directly upward to reach the cranial pole, and finally passes backward into the *prominentia cochlearis superior* (Voit) (figs. 1, 6 and 7) as the cranialmost border of the pars cochlearis, which roofs the coiled part of the cochlea (fig. 8), is called. Above the cranial pole the cartilago *supracochlearis* appears (fig. 3).

The lateral surface of the pars cochlearis (fig. 6) is smooth and gently convex in its ventral portion, and here presents a shallow groove, lying between the promontory and the cranial pole,

though it falls a little short of reaching either of these extremities (fig. 6). It is known as the sulcus caroticus (Voit) and contains a portion of the internal carotid artery (fig. 13). The sulcus caroticus does not correspond to the line of attachment of the lamina spiralis within, but crosses its cranio-ventral convex portion. Its lower part forms a low rounded projection into the lumen of the uncoiled portion of the cochlear duct, which appears in the figure of the cast of the cavity (fig. 8) as a shallow fossa.

The dorsal part of the lateral surface is made up principally by the outer wall of the vestibular portion, and forms the medial wall of the ventro-lateral otic recess. Above, the outlet of the facial foramen is to be seen, bridged by the suprafacial commissure. Below this opening is a small groove, the sulcus facialis (Voit), for the facial nerve, and below this, again, appears the elongated, crescentic, fenestra vestibuli, lying in a general direction from above downward and backward, and presenting a concavity downward and forward. It contains the anlage of the footplate of the stapes, which, however, fills only a small portion of the space of the fenestra, the remainder being occupied by the connective tissue representative of the annular ligament of the base of the stapes. Below the fenestra vestibuli is the cartilaginous septum which separates it from the fenestra perilymphatica below. This septum, which acts as a commissure to join the promontory of the pars cochlearis with the ventral surface of the pars canicularis, has been referred to by Voit as the promontorium (fig. 6).

Passing below the lower, downwardly concave, border of the promontorium we come upon the small caudal surface of the vestibular portion (fig. 2), marked centrally by the large fenestra perilymphatica, which will later be separated by the processus interperilymphaticus into the larger lateral fenestra cochlearis or rotunda (over which is stretched the membranous anlage of the membrana tympani secundaria), and the smaller, medial foramen for the aquaeductus cochleae, within which may be seen the saccus perilymphaticus (Voit). The interperilymphatic process, more prominent on the left side than on the right, has been referred to, and appears as a short, conical

projection directed forward from the inferior utriculoampullary prominence. The cochlear fenestra is apparently closed off on the left side of the Hertwig model, and almost so on the right.

The perilymphatic fenestra (fig. 2) is sharply concave, from before backward, the direction of the concavity being downward. Its inner wall is formed by the lower edge of the *massa pyramidalis* of the median wall of the vestibular portion. When regarded from below the circumference of the perilymphatic fenestra appears to have been formed by the bifurcation of the inferior cochlear prominence at the promontory, the lateral limb forming the promontorium; the medial the lower border of the *massa pyramidalis*; the two limbs uniting dorsally in the inter-perilymphatic process.

The boundaries of the medial surface have already been noted (fig. 7). It is quite smooth, and is more flattened than the lateral. Ventrally the elongated, narrow, crescentic line of union with the basal plate may be seen; immediately ventral to and parallel with this, the everted, narrow, extra-cranial surface, formed by the medial aspect of the inferior cochlear prominence, makes up the outer wall of the ventral basicochlear groove (fig. 2) as a strip 1.5 mm. wide.

Dorsal to the basal lamina the medial surface is intracranial, the strip immediately bordering the lamina being concerned in the formation of the outer wall of the dorsal basicochlear groove (fig. 5), which is sharply marked throughout, but more so above than below. In the dorso-cranial area of the medial surface the large, deep, meatus acusticus internus appears (fig. 7); below, the surface passes into the caudal surface of the *pars cochlearis* and behind into the medial surface of the *pars canalicularis*.

If we now consider, briefly, the passageway of the ductus cochlearis (figs. 8 and 9) we find the first, or uncoiled part, outwardly deflected at and for a short distance beyond its entrance from the perilymphatic fenestra. The lateral wall is here quite thin, but the opposite medial wall presents a pronounced conical thickening, to which reference has been made as the *massa pyramidalis* (fig. 2). The apex of the pyramid projects laterally into the first portion

of the cochlear duct, and, indeed, it is owing to this circumstance that this part of the duct is thrust outward to form the promontory on the outer surface. From a point just ventro-cranial to the tip of this pyramid a small commissure, known as the *commissura laminopyramidalis* (fig. 8), springs to join the lamina spiralis which is immediately ventral, and this commissure passes over the uncoiled part of the cochlear duct; at the same time it divides the *crescentic fissure* in the floor of the internal acoustic meatus into ventral and cranial parts. The caudo-ventral surface of the pyramidal mass forms the medial wall of the first part of the cochlear duct; the cranio-ventral surface constitutes the medial and steepest part of the floor of the internal acoustic meatus, while the border between these delimits laterally the slit-like foramen, piercing the ventralmost part of the meatus, which transmits the cochlear division of the acoustic nerve. Dorsally the base of the pyramid is seen to be pierced from above downward and backward by the foramen singulare (fig. 9), which leads into the cavity of the ampulla of the posterior semicircular canal, the region of exit appearing as an indentation of the inner wall just medial to the inner edge of the fenestra perilymphatica. It appears in the cast of the cavity of the capsule as a projection (fig. 9). A small portion of the dorsal side of the pyramid is concerned in the formation of the ventral wall of the vestibular space; the remainder, together with its border joining the cranio-ventral surface, is directly continuous with the cartilage of the medial wall of the vestibular part of the pars cochlearis.

The ductus cochlearis, shortly after passing the level of the lamino-pyramidal commissure (fig. 8), emerges from the vestibular part of the pars cochlearis, and enters the ventral, completely enclosed pars cochlearis (*sensu stricto*) which contains its coiled part. The only entrances into the closed portion of the cochlea are the passageways for the cochlear duct and the cochlear root of the acoustic nerve. The medial wall is here quite thin, while the lateral wall presents the coiled lamina spiralis (fig. 5).

If the suprafacial commissure were removed (fig. 7) it would be seen that the superior cochlear prominence is continued downward and backward as the first and widest part of the spiral lamina, which here separates the upper coiled portion of the cochlear duct, in front, from the internal acoustic meatus behind. In front of the upper part of the ventro-medial edge of the lamina spiralis, at a point marked by the widened cranio-ventral extremity of the foramen for the cochlear root, the medial downward continuation of the superior cochlear prominence passes over upon the medial surface of the pars cochlearis. When the medial wall of the pars cochlearis is removed it is seen that the lamina spiralis is attached to the lateral wall of the pars cochlearis, the line of attachment being in the form of a helix, which makes but little more than one turn. If the lower edge of the internal acoustic meatus (figs. 5 and 7) be followed forward and inward it passes over the upper edge of the foramen for the cochlear root to reach the medial edge of the lamina spiralis; thence it may be followed along the edge of the narrowing lamina, whose curvature becomes progressively sharper, ending on the lateral wall in a downward turn. In this way there is formed a commodious recess for the upper coiled part of the cochlear duct and its surrounding space. In all the cochlear duct makes about two turns (fig. 8).

The internal acoustic meatus (figs. 5 and 7) presents a rounded border, although its edges are somewhat straightened below and behind. The dorsal portion of the upper border is sharp, and represents the medial edge of the foramen faciale. Passing caudally the edge becomes less sharply marked on the dorsal side, the cartilaginous surfaces which form it here meeting at a right angle. Ventrally we come upon the caudal edge, which is very sharp indeed, and represents the upper edge of the base of the massa pyramidalis. The dorsal and caudal edges form a rounded angle, and about 1 cm. below this point the entrance of the foramen singulare appears. Passing upward from the ventral end of the lower edge we come upon a crescentic and illdefined border which delimits the meatus cranio-ventrally,

and passes dorsally into the medial edge of the suprafacial commissure.

The floor of the meatus is composed of three distinct portions; the ventral, formed by the first part of the lamina spiralis, the dorsal, which is the ventral edge of the wall of the vestibular portion in this region, and the medial, formed by the cranio-ventral surface of the pyramid, as we have seen. These surfaces increase in steepness in the order mentioned, so that the crescentic fissure (fig. 7) formed by their approximated deep edges, is deeper caudo-ventrally than cranio-dorsally. Looked at from within the ventral and dorsal are the only surfaces visible, the ventral presenting much the greater area. The borders of the latter where they join with the margins of the meatus present the enlarged extremities of the crescentic fissure, the upper of which serves for the passage of the facial nerve, the lower and anterior for the upper part of the cochlear division of the acoustic nerve.

Five foramina appear in the internal acoustic meatus, and of these the foramen singulare has been considered. It transmits the posterior ampullary nerve to the inferior cribriform macula. The other four are parts of the crescentic fissure. This latter is divided into almost equal limbs by the lamino-pyramidal commissure, which has been noted overlying the first part of the cochlea, and joining the dorsal surface of the first part of the spiral lamina with the pyramid. The ventral limb is long and slit-like, widest in its ventro-cranial end, and transmits the fibres of the cochlear root of the acoustic nerve. It will later become the spiral foraminous tract. The cranial limb is separated by two cartilaginous septa into three foramina, the upper, which we have seen, being large, and transmitting the 7th cranial nerve, and being known as the facial foramen, the lower two being of about the same size, and transmitting the superior and inferior branches of the vestibular root of the acoustic nerve (figs. 5 and 7).

I have also reconstructed a model of the cavity of the otic capsule, and from the illustrations of this a conception of the general plan of the cavity may be gained (figs. 8 and 9). This

cast includes not only the membranous labyrinth but the space surrounding it, together with the entrances of the various foramina. In the illustration the laminopyramidal commissure appears as a foramen behind the coiled portion of the cochlear tract. In general form the cast resembles the later osseous labyrinth.

The cartilago supracochlearis (figs. 1, 3, 13) may now be considered. This is a small, rounded mass of cartilage, situated upon the cranial pole of the pars cochlearis, and, in the model, is about 8 mm. wide, and almost as long dorso-ventrally. It is quite free from cartilaginous union with the underlying cochlea, but the two are more closely approximated posteriorly than anteriorly, where the intervening connective tissue is thicker. The cartilage is immediately beneath the anterior part of the semilunar ganglion (fig. 13), and the material of which it is formed is mature cartilage of apparently the same age as that in the adjoining pars cochlearis.

It is difficult to say what may be the significance of this cartilage. Certainly it cannot be any one of the Restknorpeln which Voit describes in his Stage II (43 mm.) of the rabbit, since only one of these, Restknorpel b, corresponds at all in position with this cartilage, but it is distinctly above the semilunar ganglion while the supracochlear cartilage is below it. I am inclined to regard it as a rudiment of the commissura (or trabecula) aliochlearis, which Jacoby describes in his 30 mm. human embryo as a cartilaginous bridge extending between the anterior part of the pars cochlearis of the otic capsule and the ala temporalis. There is no evidence in my model of such a commissure, though the surfaces of the processus alaris of the temporal wing and the ventral surface of the pars cochlearis are very close together, and in the later stage modelled by Hertwig (80 mm.) there is no evidence of either commissure or rudimentary cartilage in this location, indicating that the cartilage in my embryo is probably undergoing retrogression. Voit describes and figures such a commissure in the skull of the rabbit, which encloses the carotid foramen laterally. He states it is a direct forward continuation of the planum supracochleare of the pars cochlearis.

The parietal plate (fig. 3) is a thin, semi-crescentic plate of cartilage, situated above and behind the pars canalicularis of the otic capsule, and bearing upon its median surface a concave impression for the brain (fig. 5). The ventral extremity is wide, and is surmounted by an irregularly formed and rudimentary portion, the upper border of which is overlaid laterally by the caudal edge of the parietal bone. Above this part, and lying in the membrane within the parietal bone is to be seen, on the right side, an elongated nodule of cartilage, which may be known as the *cartilago cranii lateralis* (fig. 3)—probably a remnant of the side wall in this region. On the left side there is a somewhat smaller nodule. That this portion is undergoing retrogression is evident from a comparison with the models of Levi, on the one hand, and with the model of Hertwig on the other, when it is seen that the 14 mm. stage of Levi marks, perhaps, the stage of greatest development of the parietal plate, there being, after this, a progressive reduction, moderate in the 28 mm. stage of Levi and in my model, and pronounced in that of Hertwig.

With the otic capsule the parietal plate is connected at two points—in front through the capsuloparietal commissure, and below this through the bridge of cartilage between the capsuloparietal and capsulooccipital fissures. The ventral edge is indented and presents no evidence of the sphenoparietal commissure, such as exists in certain of the lower mammals, as the rabbit and pig, and which represents, according to Gaupp ('00) the taenia marginalis of reptiles. The upper border is concave upward, and in its ventral portion there may be seen a small incisure, open behind, formed by an overhanging, backwardly projecting spicule from the uppermost part of the plate. This incisure appears to be the representative of what Mead calls the *fissura laminae parietalis* in the skull of *Sus*, where it is quite conspicuous. The upper border is continuous above with the membrane covering the brain.

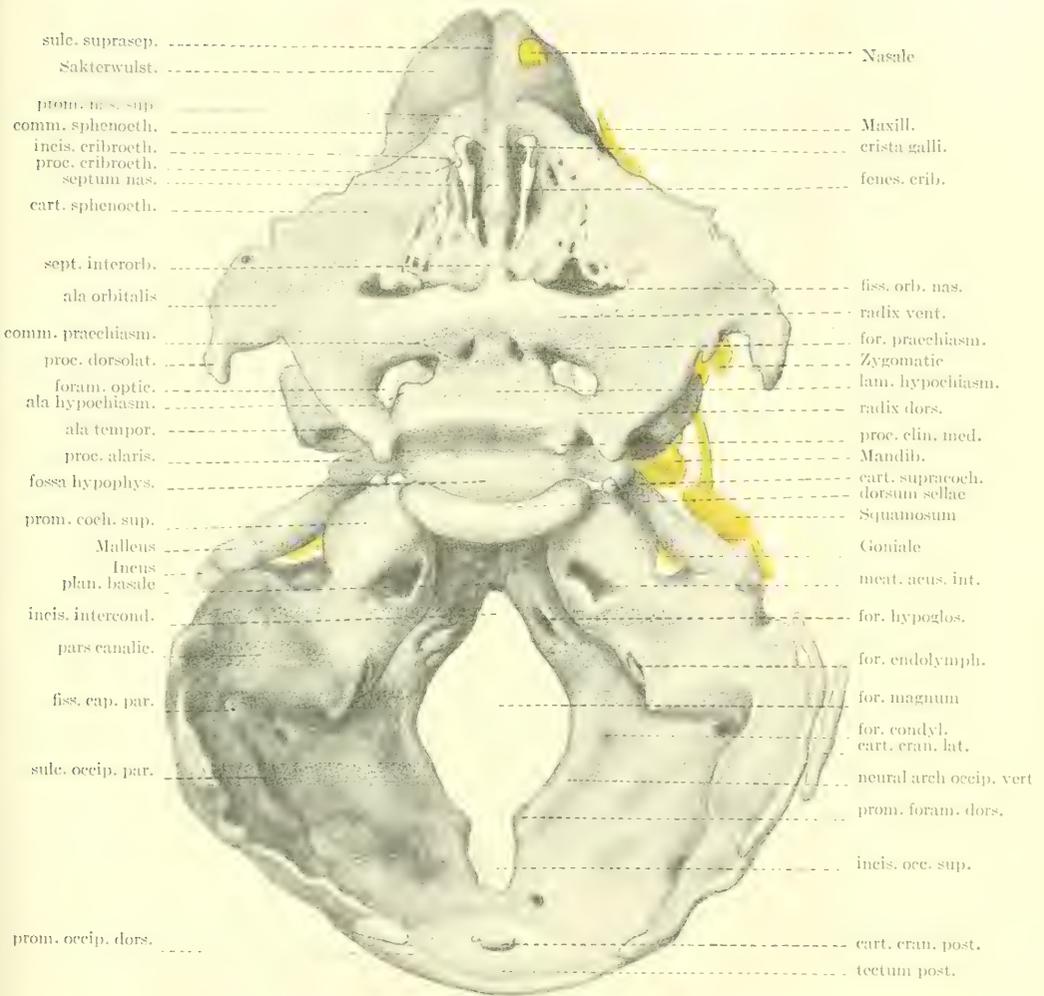
The lower border is continuous ventrally with the otic capsule at the upper edge of the capsulooccipital fissure. Behind this it follows the elongated occipitoparietal groove (figs. 1 and 5) which runs backward to the dorsal occipital prominence, and

marks the zone of union with the squama. Several small blood-vessels are found in the ventral portion of the occipitoparietal groove, but in its dorsal part there is but a single small vessel. Just above the groove, and running parallel with it, is a low rounded ridge. The dorsal, scimitar-like extremity of the parietal plate is shown projecting freely dorso-medially. The outer surface is convex and is but indistinctly marked off from the underlying squama.

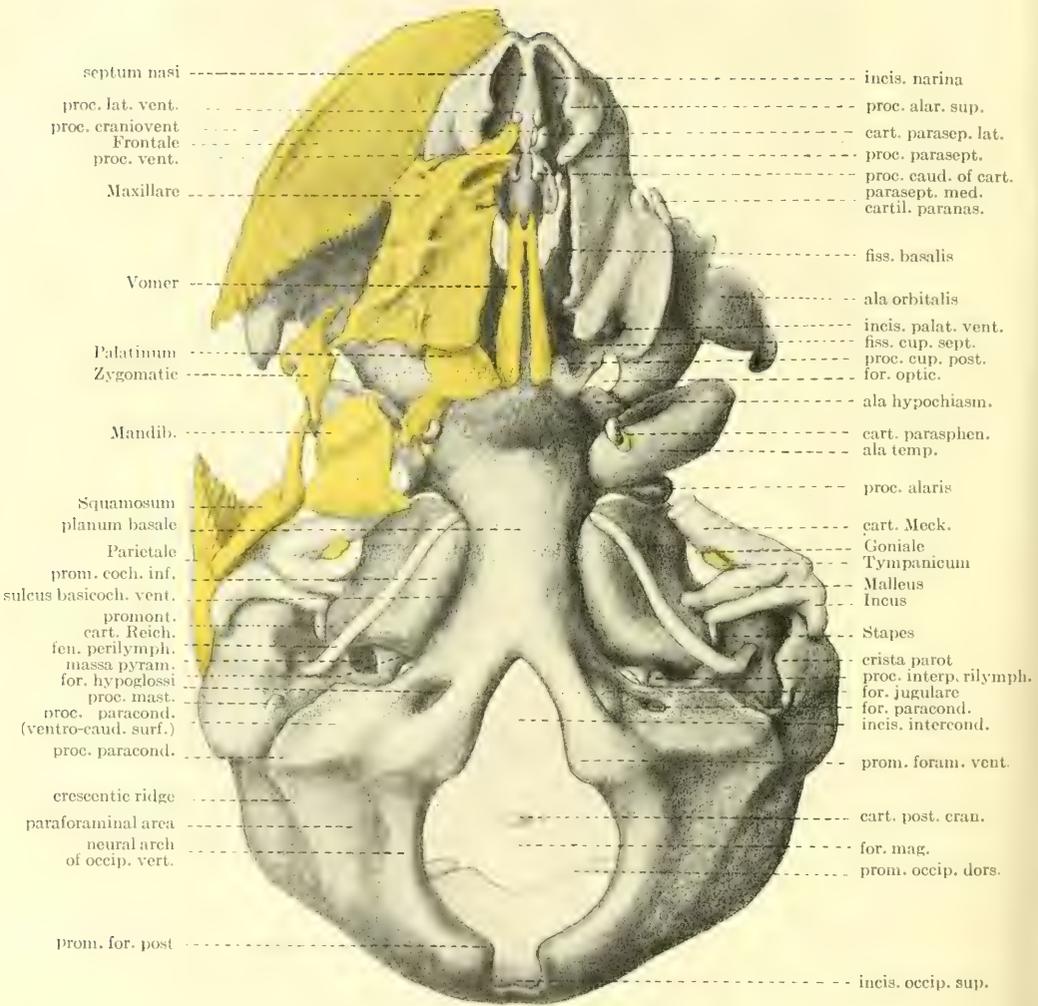
In the membrane forming the posterior and superior part of the cranium, considerably above the tectum posterius, are to be seen two small cartilages lying side by side, the cartilaginee cranii posteriores. The cartilage on the right side, though small, is relatively very large when compared with its partner of the left, which is insignificant.

These cartilages appear to represent the unpaired mass described by Bolk as lying above the tectum synoticum, which subsequently disappears. Possibly in my embryo they are undergoing reduction. Mead describes a somewhat similar small free nodule in *Sus*, but this is single and, although in the midline, it lies immediately above the tectum. He calls it the processus ascendens of the tectum posterius, and thinks it may possibly be the homologue of the processus ascendens of the tectum posterius of reptiles.

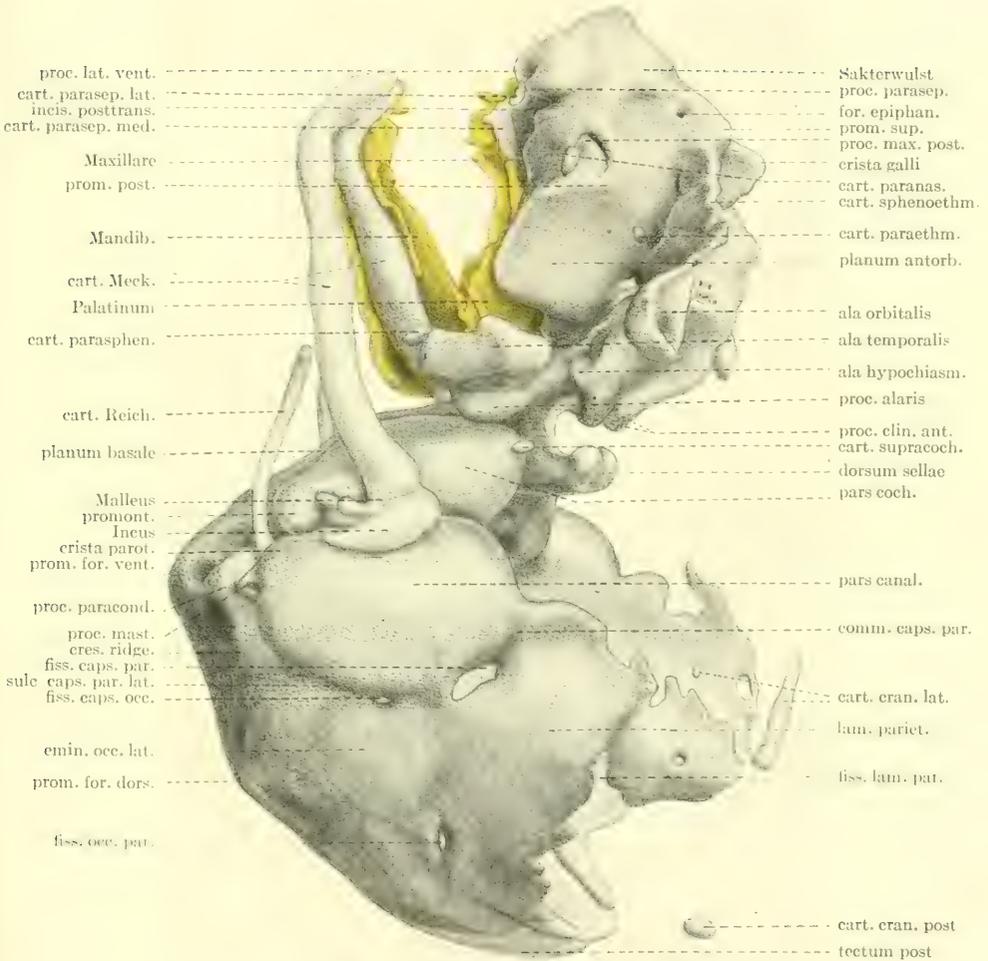
PLATES



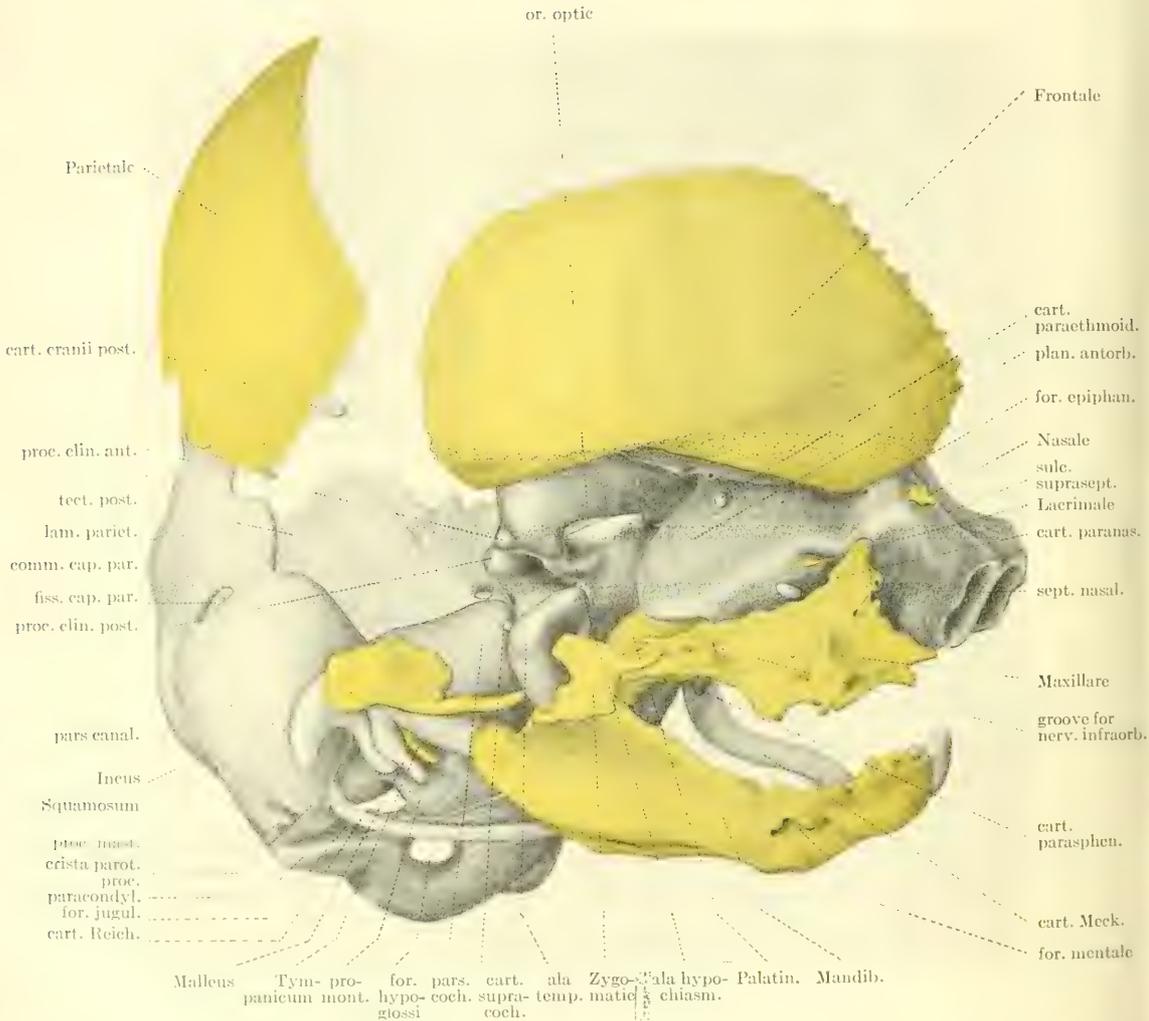
1 Wax plate reconstruction of the chondrocranium of a 40 mm. human fetus, seen from above.



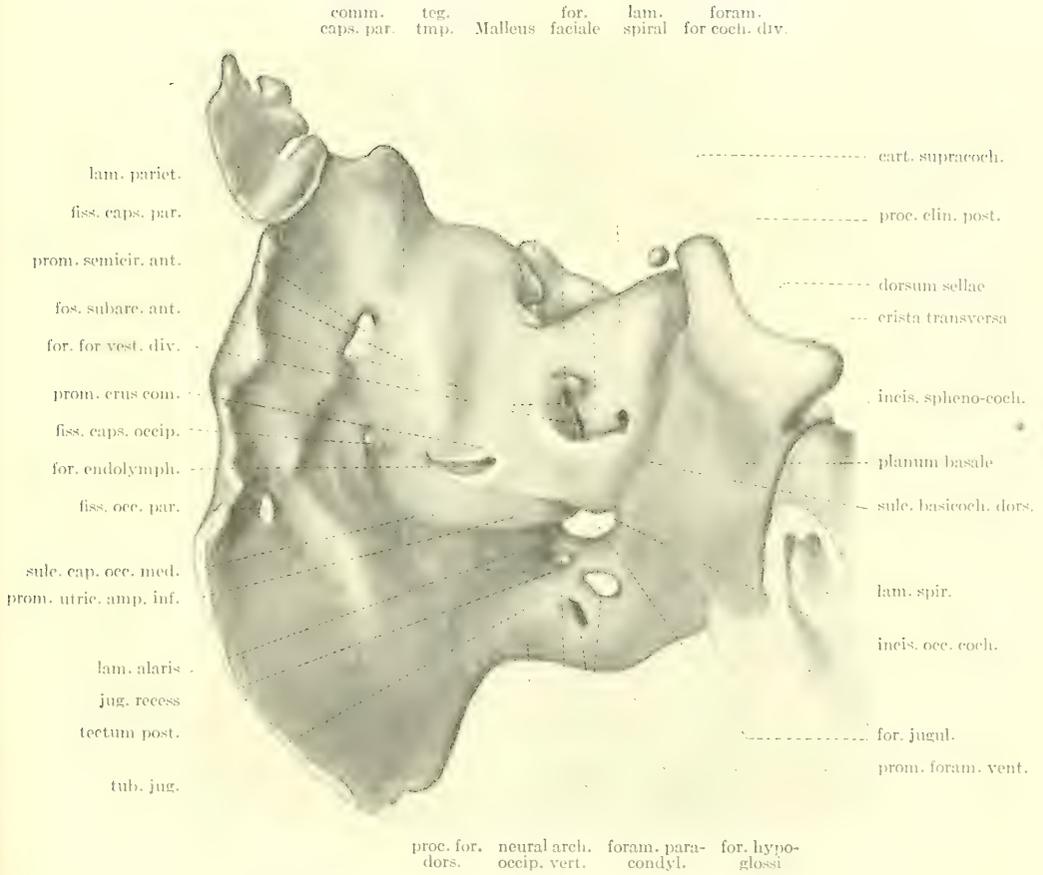
2 Skull seen from below.



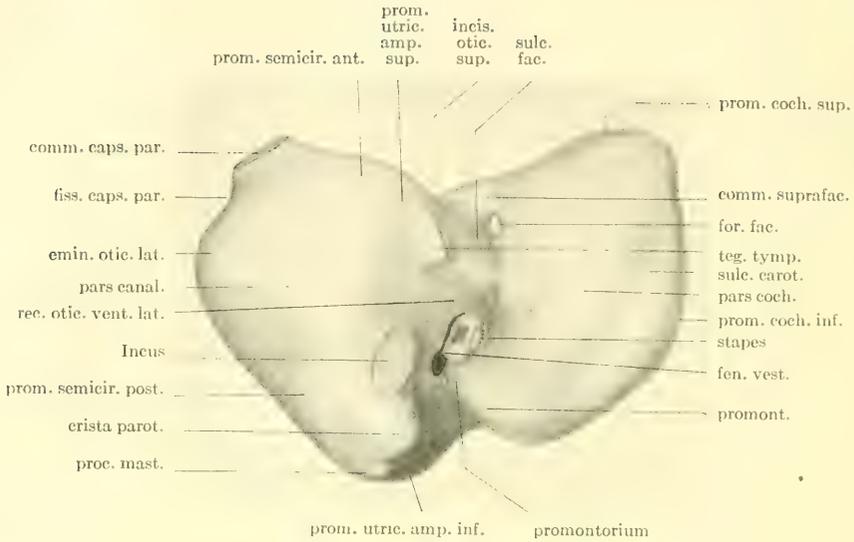
3 Skull seen from left side.



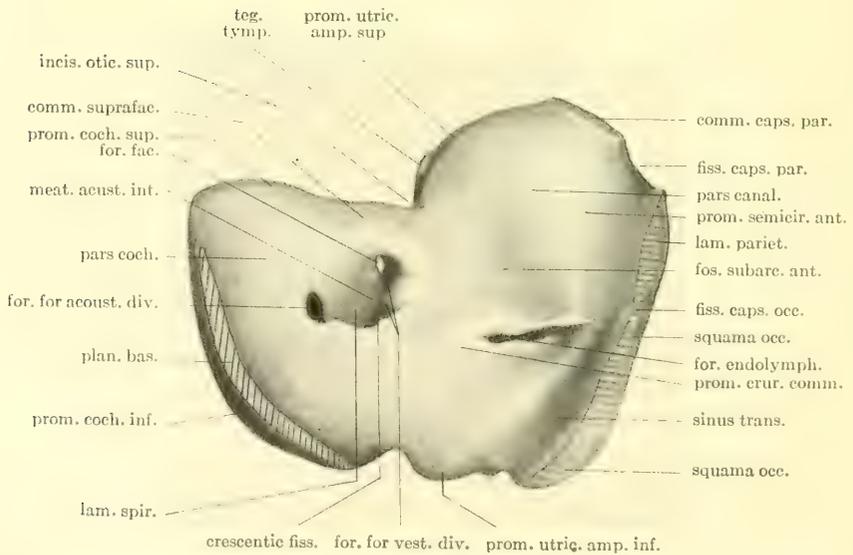
4 Skull showing the right anterior aspect.



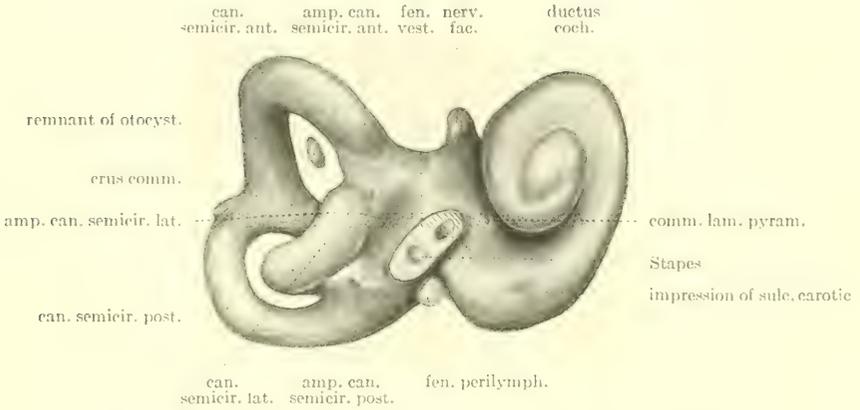
5 The left otic and occipital regions, viewed from within.



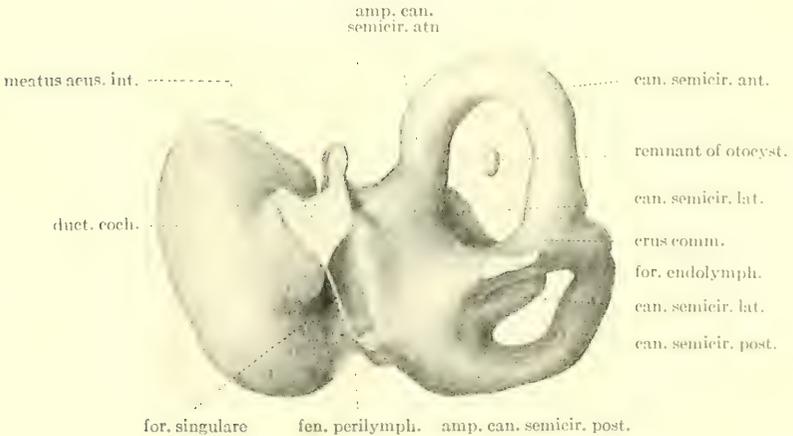
6 Lateral aspect of right otic capsule.



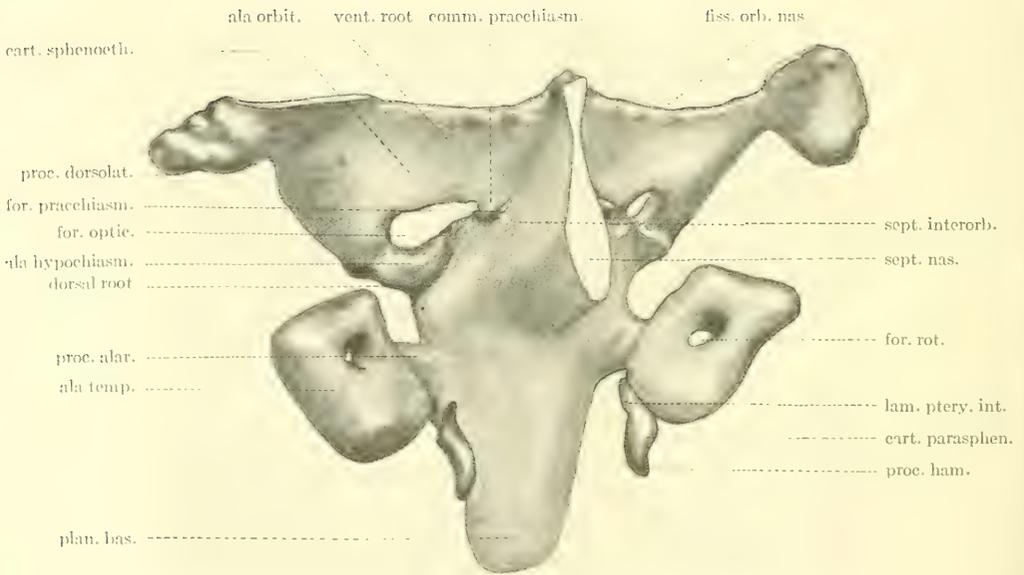
7 Medial aspect of right otic capsule.



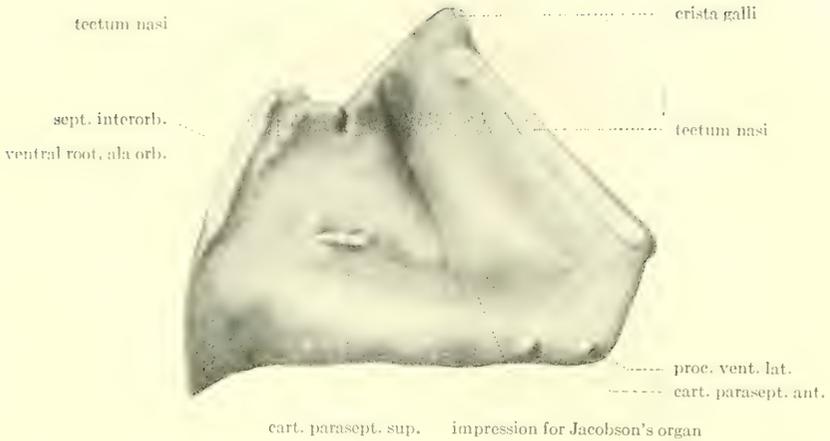
8 Lateral aspect of cast of cavity of the right otic capsule.



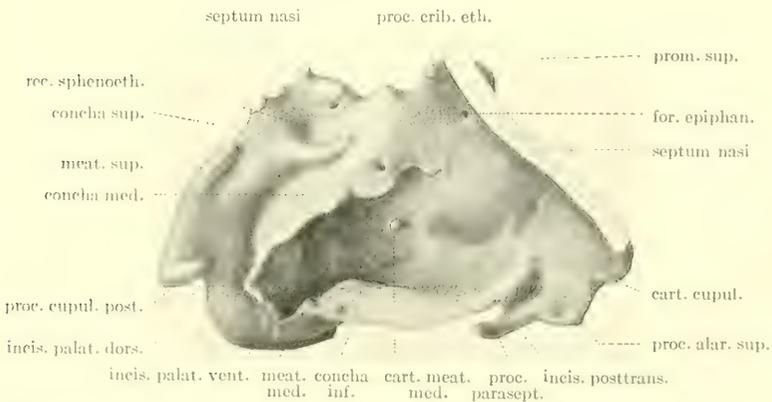
9 Medial aspect of cast of cavity of the right otic capsule.



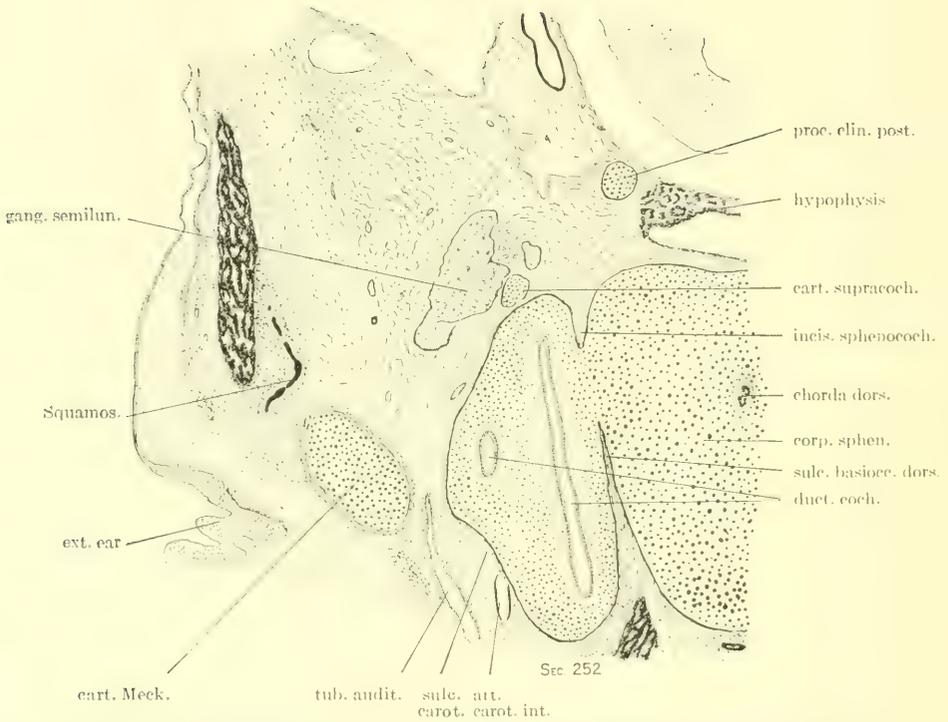
10 Antero-lateral aspect of detached sphenoidal region.



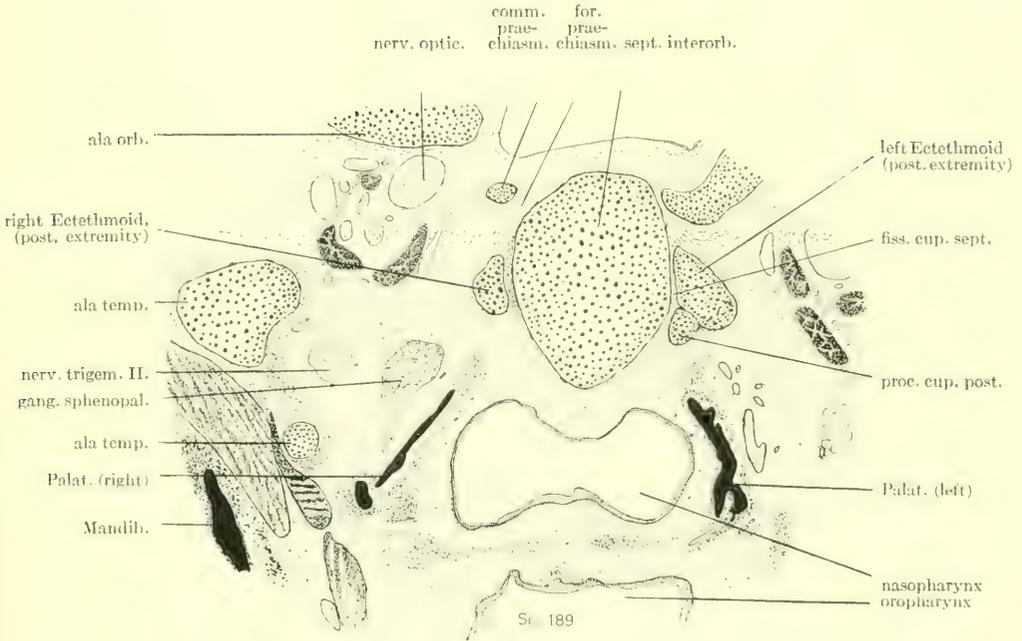
11 Shows the nasal and interorbital septa from the right side. Unions with ventral root of ala orbitalis, tectum nasi of ectethmoid, ventro-lateral process, and anterior paraseptal cartilage are seen. The superior paraseptal cartilage appears in the more dorsal area. The dorsal cut surface of the interorbital septum could not be represented from this position, but it begins above a little below the level of the lower edge of the ventral root of the ala orbitalis. Dorsal to this root the posterior edge of the illustration represents the dorso-cranial border of the septum interorbitalis.



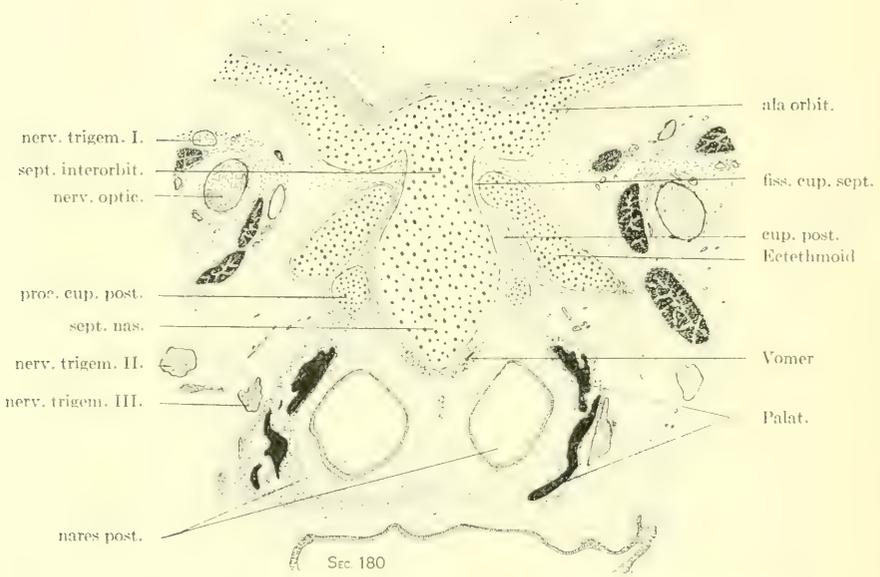
12 Medial aspect of left ectethmoid. Unions with the nasal septum are shown.



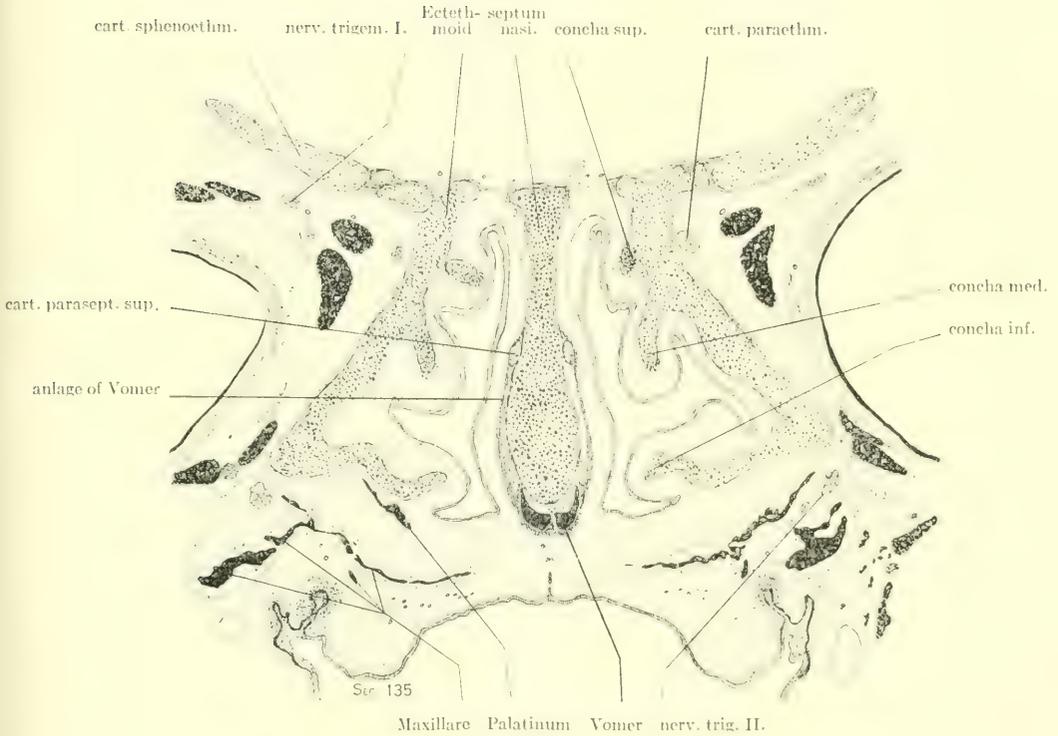
13 Coronal section through head at level of hypophyseal fossa, showing the cartilago supracochlearis overlaid by the semilunar ganglion.



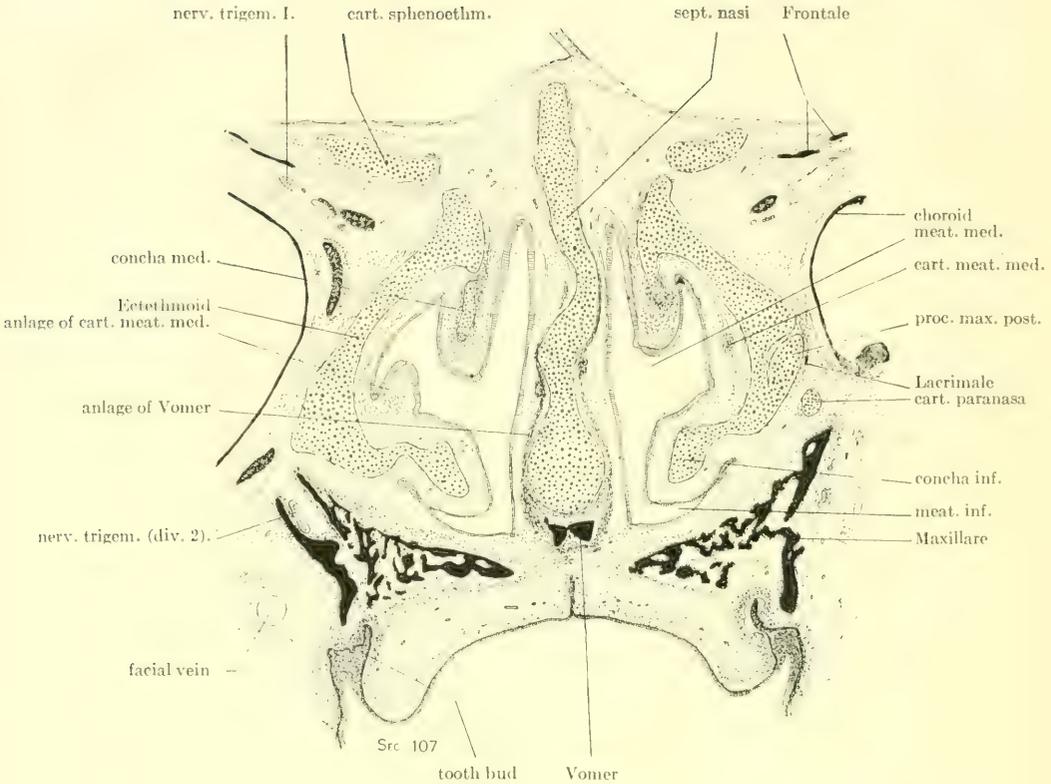
14 Coronal section through head showing dorsal part of ethmoidal and ventral part of orbitotemporal region. Asymmetry is due to obliquity of section. The posterior cupular process on the left side (right side of illustration) is shown joining with the side wall; on the other side, in which the plane of section is posterior, the process is completely joined to the ectethmoid.



15 Coronal section through the dorsal part of ethmoidal region, showing posterior cupular process, the nasal septum, the interorbital septum with attached alae, and the palatine and vomer.

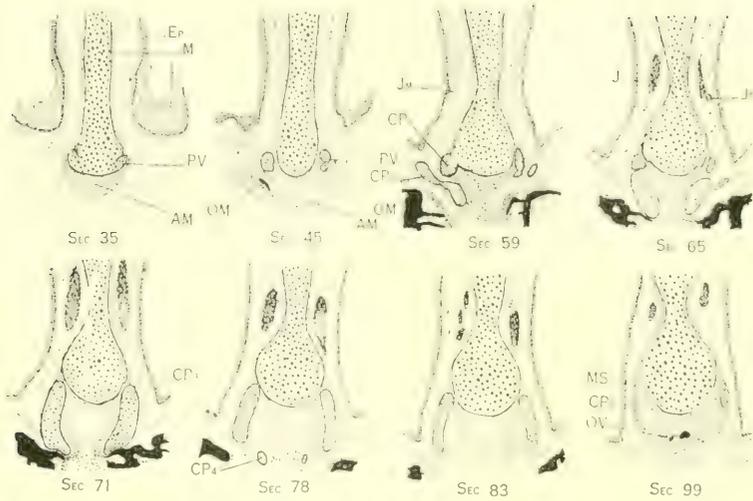


16 Coronal section through the ethmoidal region, showing superior paraseptal cartilages, paraethmoidal cartilages, the vomer, maxilla, and palate bones.



17 Coronal section through ethmoidal region, showing nasal septum, ectethmoid, with attached conchae, cartilago paranasalis, and vomer, maxilla and lacrimal bones.

- AM—Anlage of the maxilla
- CP₁—Cranio-ventral process of medial lamina of anterior Jacobsonian cartilage
- CP₂—Lateral lamina of anterior Jacobsonian cartilage
- CP₃—Body of medial lamina of anterior Jacobsonian cartilage
- CP₄—Caudal process of medial lamina of anterior Jacobsonian cartilage
- CP₅—Dorsal extremity of lamina of medial anterior Jacobsonian cartilage
- Ep—Epithelium of nasal cavity
- J—Organ of Jacobson (right)
- Jd—Duct of left organ of Jacobson
- Jm—Meatus of duct of right organ of Jacobson
- M—Mesethmoid
- MS—"Suspensory membrane" of Fawcett, the anlage of the vomer
- OM—Maxilla
- OV—Vomer
- PV—Ventro-lateral process



18 Series of eight coronal cross-sections, through caudal portion of mesethmoid, in the region of the anterior parasseptal cartilages. Sections numbers 35, 45, 59, 65, 71, 78, 83, and 99. Magnif.

Section 35 shows the ventral end of the ventro-lateral process, *PV*, and its connection with the caudal border of the mesethmoid. In section 45 the dorsal ends of these processes are shown in cross section, with the ventral extremity of the maxilla *OM*; the membranous anlage of this bone appears in sections 35 and 45—*AM*. Part of the epithelium of the nasal cavity is represented in all the sections *Ep*. Section 59 is through the ventral end of Jacobson's cartilage and shows the cranio-ventral process of the medial lamina *CP*₁ on the right side attached to the mesethmoid, while the outer lamina, *CP*₂ lies free in the mesenchyme. Owing to the section having been cut somewhat more deeply on the right than on the left side, the left side of the illustration shows a somewhat more dorsal plane than does the right. The meatus of the right Jacobsonian duct opens at *Jm* in section 59. Section 65 shows, on the right side, the dorsal tip of the left outer lamina of Jacobson's cartilage, and medial to this the main part of the inner lamina below, and the cranio-ventral process above. The organ of Jacobson appears in this and following sections, and it will be observed that the mesethmoid has become quite thin in its vicinity, the organ lying in a concavity of the cartilage. In section 71 the main lamina of Jacobson's cartilage is seen *CP*₃, and it will be noted that at its caudal extremity the relationship to the maxilla is very intimate. In the next section, No. 78, the caudal process is seen separated from the main mass of the cartilage, and in the next section, No. 83, this process has disappeared. In No. 99 the dorsal tips of the Jacobsonian cartilages are seen *CP*₅, and below the mesethmoid, lying in a mass of condensed mesenchyme, the anterior tips of the vomer are seen as two small spicules of bone.

II. THE SKULL OF A HUMAN FETUS OF 40 MM.¹

CHARLES CLIFFORD MACKLIN

James H. Richardson Fellow in Anatomy, University of Toronto

Regio orbitotemporalis

The orbitotemporal region, in the present stage, is composed of the cartilage of the sphenoidal anlage and unites the otic region, behind, with the ethmoidal region in front. In it we recognize an unpaired, median portion, made up of that part of the central stem of the chondrocranium which contains the bend; this represents principally the cartilaginous body of the sphenoid, and is directly continuous dorsally with the otic portion of the planum basale and ventrally with the nasal septum of the ethmoidal region. In addition there are two paired, lateral parts, the forerunners of the greater and lesser wings. The internal pterygoid plate, which is laid down in membrane bone, will be considered in the section devoted to the discussion of the purely osseous elements.

If we examine, successively, the parts of the median portion, beginning dorsally, we note first a prominent transverse ridge, the crista transversa (fig. 5), which marks the boundary between the orbitotemporal and otic portions of the median stem. Directly continuous with this ridge, and springing upward from it, is the prominent dorsum sellae (figs. 1 and 3), here showing no median perforation, as it does in the rabbit (Voit), and it is owing to this circumstance that the upper edge of the crista is, in homo, entirely obliterated.

The dorsum sellae forms the conspicuous posterior wall of the hypophyseal fossa. Its upper and lateral corners are thickened

¹Part I of this paper was published in the July number of the Journal, vol. 16, no. 3.

and project upward, outward and forward in a horn-like manner, so that their ventral extremities overlie, to some extent, the hypophyseal fossa. These are the posterior clinoid processes. Their upper extremities rise higher than the cranial edge of the dorsum sellae between them, and hence the latter is concave cranially, as well as ventrally. The dorsal surface of the dorsum sellae is gently concave from side to side, and from above downward, passing uniformly over upon the surface of the otic portion of the planum basale below.

Histologically there is here, at this stage, no evidence of the primary separation of the chondrous anlagen of the dorsum sellae, such as Fawcett describes in earlier stages (19 and 21 mm.), except a slightly younger condition of the cartilage at the ventral side of the junction of the dorsum sellae and crista transversa.

In front of the dorsum sellae we come upon the wide, flattened floor of the hypophyseal fossa (fig. 1), or sella turcica, which opens laterally into the side-parts of the middle cranial fossa, and is thus more correctly a short wide groove than a fossa. Ventrally the wall rises abruptly,—almost vertically,—to reach a transverse ridge,—the anlage of the tuberculum sellae. The lateral edge of the ventral wall presents, on the right side, but not on the left, a small, conical backwardly projecting middle clinoid process. The tuberculum sellae of my model is much more prominent than it is in that of Hertwig. It is interesting to note in passing that Levi is of the opinion that in the development of the human skull the sella turcica is the only part to retain its primitive position, the other parts moving cranially, and he finds in the 28 mm. stage, among other evidences of this, the appearance of the tuberculum sellae as the anterior wall of the sella turcica. There is no evidence of the tuberculum sellae in the 17 mm. stage, while in the 28 mm. stage the sella has a vertical ventral wall, as in my model.

The part of the central stem of the chondrocranium composing the floor and ventral wall of the sella turcica has been termed the Balkenplatte or lamina trabeculi. It is wide, and, when compared with the parts in front and behind it, quite thin. It shows no evidence of perforation. From the ventral half of each

lateral edge of the floor there is seen to project, in a direction downward and outward, the short, rod-like *processus alaris*.

When the model is regarded from the side (fig. 3), the floor of the sella turcica, which is slightly concave cranially, appears as the upper expanded end of the *planum basale*, the ventral wall of the sella appearing as a continuation upward of the ventral surface of the *planum*; indeed if the ventral border of the latter (which we have noted is almost straight) is projected upward it will pass just ventral to, and parallel with, the ventral wall aforementioned. From the same position, too, the ventral wall of the sella appears as the dorsal expanded end of the cranio-ventral or horizontal limb of the central stem of the chondrocranium, and the appearance is as if the caudo-dorsal edge of this had been applied to the cranio-ventral edge of the vertical limb, making an angle of 115° , open caudo-ventrally, the flattened extremities forming the ventral wall and floor of the sella turcica respectively. Ventrally (fig. 2) this angle is seen to be quite sharply marked, and to lie on the line between the ventral edges of the roots of the *processus alares*, or, just below the level of the ventral wall of the sella. Ventral to this angle the cartilage of the central stem gradually becomes narrower from side to side, and expanded caudo-cranially, passing ventrally into the nasal septum (fig. 10).

If we now turn our attention again to the upper surface of the median stem, we pass forward from the sella turcica, over the rounded, transverse, *tuberculum sellae*, and come upon the flattened *lamina hypochiasmatica* (fig. 1) (*suleus chiasmaticus* of Levi), triangular in shape, with the apex placed ventrally in the midline, and the base formed by the upper edge of the ventral wall of the sella turcica. This surface underlies the optic chiasma, and is horizontal, thus making almost a right angle with the ventral wall of the sella. Ventrally the apex rises upon the dorsal edge of the interorbital septum, forming therewith an angle of 120° . Immediately lateral to this junction is a small, slit-like foramen, which may be known as the *foramen praechiasmaticum* (figs. 1, 10 and 14); it has been shown in the models of the skulls of several mammals, as the ape (Fischer) and the rabbit (Voit),

as well as man (Hertwig). It contains nothing but loose connective tissue. It does not appear in Jacoby's figure, but the optic foramen extends all the way to the interorbital septum, and one may assume from this that the small isthmus of cartilage, which cuts off this small aperture from the optic foramen, and which may be known as the *commissura praechiasmatica* (figs. 1, 10, 14), has been developed between the 30 and 40 mm. stages. In my sections this commissure shows a rather younger condition of cartilage than that found in the surrounding chondrocranium. The foramen praechiasmaticum evidently disappears later, as it is not to be found in the osseous condition. At the dorsal extremity of the optic foramen the lamina hypochiasmatica is seen to pass over upon the dorsal root of the *ala orbitalis*.

A feature which I have not noticed in the description of human primitive skulls, but which is described by Voit in the skull of *lepus*, as the *ala hypochiasmatica*, is a small but strong crescentic ridge which projects antero-laterally from the surface of the lamina hypochiasmatica just ventral to the origin of the dorsal root of the *ala orbitalis*, and which is continuous dorsally with this root. It appears in figure 10 and may be seen from above as a projection into the optic foramen (fig. 1). It presents a convex ventral edge, and is separated from the surface of the interorbital septum, lying within, by a distinct furrow containing only connective tissue. In the Voit model of the skull of rabbit this shows beginning ossification, but such is not the case in my model, in which the *ala* presents a somewhat younger type of cartilage, especially in the ventral edge, when compared with that of the adjacent cartilage.

Ventrally, as we have seen, the lamina hypochiasmatica is continuous, medially, with the dorsal border of the interorbital septum. From the sides of this septum the ventral roots of the *alae orbitales* are seen to spring (fig. 1), and its cranialmost edge, delimiting the orbitonasal fissure medially, passes over directly upon the nasal septum.

The interorbital septum (figs. 10, 11, 14 and 15) is narrow cranially, being delimited dorsally by the roots of the *alae orbi-*

tales and ventrally by the dorsal extremities of the side-walls of the ethmoidal region (fig. 11). It is thicker below than above, its lower surface being keel-like, and passing horizontally forward upon the lower edge of the nasal septum; the upper part becoming increasingly thinner as we follow it ventrally, to coalesce with the upper portion of the same structure. It represents a transition from the flattened *Balkenplatte* to the thin nasal septum, in which the median stem appears to undergo a torsion of 90° , and forms the median delimitation of the narrow inner recess of the orbit.

The interorbital septum of man is homologous with the structure bearing the same name found in the Saurians, but, as is the case in the other mammals, it is rudimentary when compared with these lower forms. A theory which seeks to account for the shortness of this septum when compared with the condition in the lower forms is that, in the mammals, the nasal side-wall has gradually grown backwards, to encroach more and more upon the territory of the interorbital septum, to the advantage of the nasal septum (Mead).

If we start at the floor of the sella turcica (fig. 1), and proceed upward and forward we come upon three successive steps, at about equal intervals apart, formed, as we have seen, by the lamina hypochiasmatica, the uppermost edge of the interorbital septum, and finally, in the most cranial, which reaches the highest point of the chondrocranium, by the crista galli of the mes-ethmoid (fig. 11).

The orbital wings (figs. 1 and 10) are the cranio-ventral and the larger of the paired lateral extensions of the sphenoidal anlage. Each wing has the form of an imperfectly defined, triangular plate, with the irregular base parallel with the median plane, and the apex lateral and turned dorsally. The plate is gently concave downward, forming the roof of the orbit; above it takes part in the formation of the floor of the anterior cranial fossa. Of the unions, two are with the central stem of the chondrocranium, the dorsal and ventral roots, and one which is elongated and broken by several foramina is with the side-wall of the ethmoidal region. The two wings lie almost hori-

zontally, and thus differ from the earlier conditions, where they are considerably higher laterally than medially (Jacoby).

The dorsal root is the smaller, and is directed outward and slightly upward. Thick and rounded in section, its cartilage is directly continuous with that of the lamina hypochiasmatica, from the side of which it has been seen to project. A short distance from its origin it widens out, and at the same time rises cranially to form a plate, flattened from above downward, outward and slightly forward, the dorsal edge of which presents, near the median line, a backward projection, the anterior clinoid process (fig. 3). Between this process and the edge of the Balkenplatte, rendered more sharply defined on the right side by the middle clinoid process, there is to be seen a distinct notch, which lies dorsal to the root, opposite the most posterior extremity of the optic foramen, and conveys the internal carotid artery. The dorsal border of the wing, after leaving the anterior clinoid process, passes upward, forward and outward, making a dorso-lateral convexity, and finally turns abruptly backward upon the dorso-lateral process. The ventral border of the root forms the dorsal and part of the lateral border of the foramen opticum, while its lower surface, together with that of the wing lateral to it, forms the cranial delimitation of the superior orbital fissure.

The ventral root is wide and flat, and is directed outward and slightly backward. It is considerably the longer, as well as the broader, and is directly attached to the dorsal border of the interorbital septum along the line indicated in figure 11. In addition it is connected, through the praechiasmatic commissure, with the ventro-lateral edge of the hypochiasmatic lamina (fig. 1). The ventral root occupies a somewhat higher level than the dorsal. The dorsal border curves outward and backward, to assist in the delimitation of the foramen opticum; the ventral border passes almost directly outward, and forms the dorsal border of the orbitonasal fissure. Through the portion of the wing lying lateral to the optic foramen it is continuous with the dorsal root; and in this way is formed the shelving side of a recess, the floor of which is made up of the hypochiasmatic

lamina, in which is found, among other structures, the optic chiasma.

The optic foramen is pear-shaped in outline, with the narrow end ventro-medial. The lateral border is somewhat higher than the medial, and is downwardly and inwardly concave. The optic nerve and ophthalmic artery may be seen to pass through it, the former overlying the latter.

The ventralmost portion of the orbital wing has been known by the name of cartilago sphenothmoidalis. This is a triangular plate of cartilage, somewhat thinner than that composing the remainder of the wing, and showing ventrally a connection with the superior prominence of the lateral nasal cartilage through the sphenothmoidal commissure (fig. 1), and dorsal to this connections at several points with the lateral nasal cartilage as far back as the orbitonasal fissure, the bonds of attachment being broken by intervening foramina. Thus there is formed here a secondary, lateral cribriform plate, similar to that shown in the model of Hertwig, which leads from the anterior cranial fossa, not into the nasal capsule, but into the orbit. The dorsal margin is irregular and forms the outer half of the ventral margin of the orbitonasal fissure, the medial half being formed by the dorsal surface of the ectethmoid.

The ventral border of the orbitonasal fissure is somewhat lower than the dorsal. The fissure is elongated, its long dimension being directed laterally, and in this it differs from the condition shown in the skull of *lepus* (Voit), where it is directed ventro-laterally. In the latter animal, too, there are no connections with the ectethmoid dorsal to the sphenothmoidal commissure. As in the skull of *lepus* (Voit) so in man, the fissure is principally filled with connective tissue, but in its ventral region it transmits the anterior ethmoidal nerve and vessels from the orbit to the anterior cranial fossa. It is thus, in this region, representative of the anterior ethmoidal foramen of the adult skull.

The lateral border of the ala orbitalis is serrated, and passes directly backward and outward to terminate in the hornlike, dorso-lateral process (fig. 1). It overlies, except at its tip, the

median edge of the orbital plate of the frontal bone (fig. 2). The dorso-lateral process marks the lateral extremity of the ala, and also represents the ventral rudiment of the primitive taenia marginalis of this region, which in the lower forms stretches over the foramen sphenoparietale to make a connection with the parietal plate, as has been already noted.

The ala temporalis (fig. 10) is the smaller of the paired lateral appendages of the median portion of the sphenoidal anlage. It lies lateral to the Balkenplatte, and, for the most part, in front of and below the level of the floor of the sella turcica (fig. 3). As H. Fuchs remarks one must distinguish in the temporal wing of mammals two portions; a medial, sloping steeply downward and outward, and a lateral, ascending part. The medial portion, or processus alaris, is a short, straight, rodlike mass of cartilage, directly continuous with the outer edge of the Balkenplatte, from which, as we have seen, it projects downward, outward and slightly backward. Its dorsal surface comes into close contact with the cranio-ventral pole of the pars cochlearis, but is not connected therewith by a cartilaginous bridge (commissura alicochlearis), as is the case in the Jacoby model, and in the models of several of the lower mammals. What I regard as a remnant of this bridge is, however, to be found in the supracochlear cartilage, which has already been described. Levi was unable to find any trace of this commissure in his specimens.

It may be noted in passing that Jacoby states that this bridge extends from the lateral edge of the sella turcica to the anterior edge of the ear capsule, but he has evidently included in the median portion of this the structure which other authors refer to as the processus alaris. Jacoby states that the ala temporalis springs outward from this bridge; perhaps a better way of stating it would be to say that the ala temporalis, through the processus alaris, springs from the edge of the floor of the hypophyseal fossa, and that the bridge, or commissura alicochlearis, connects the ala with the anterior surface of the pars cochlearis of the otic capsule. By the disappearance of the commissura alicochlearis the carotid foramen is left open laterally. It is quite small, being delimited ventrally by a small notch between

the root of the alar process and the Balkenplatte, and dorsally by the ventral basicochlear groove, which passes backward into the sphenocochlear notch.

The ventral surface of the alar process is almost completely taken up with the attachment of the lateral portion of the wing. The rounded outer extremity projects freely into the surrounding mesenchyme (fig. 1).

The larger lateral portion of the temporal wing lies immediately below the dorsal portion of the orbital wing, from which it is separated by the superior orbital fissure, now open laterally (fig. 4). It is rhomboidal in shape, the long axis being directed upward, outward and slightly forward, towards the lateral extremity of the ala orbitalis. The central portion of its mass is perforated, from before backward, by the large foramen rotundum, which transmits the second branch of the trigeminal nerve. The nerve, however, by no means fills the foramen, the greater portion of the space within it being occupied by connective tissue. The foramen rotundum is found in Levi's 28 mm. stage, but not in Jacoby's 30 mm. stage, the second branch of the fifth nerve here occupying a groove upon the upper surface of the wing. It is well shown in the illustrations of Fischer's ('03) ape skulls.

Immediately below the foramen rotundum the dorsal surface is concerned in the union with the ventral surface of the alar process, the long axes of the medial and lateral portions crossing at a right angle, open above. When regarded from above the effect is as if the caudo-medial corner of the lateral portion had been applied to the ventral surface of the medial portion in such a way as to leave the lower extremities of both free. The foramen ovale is not as yet formed.

Histologically there is to be seen at the junction of the alar process and lateral portion a sheet of younger cartilage and procartilage cells, which almost completely separates the two portions. This is evidently the last trace of the primitive separation of these parts, of which Levi, Fawcett and other authors speak. In the 14 mm. stage Levi finds the temporal wing represented by two procartilagenous anlagen, separated by a sheet of connective tissue.

The portion of the wing above the foramen rotundum is known as the lamina ascendens, and ends laterally in a somewhat sharp angle, which projects freely outward and forward. The uppermost angle, rather more blunt, is to be seen almost immediately under the lateral edge of the optic foramen. The dorsal surface of the lamina ascendens is convex, and terminates dorsally in a ridge, bordering the foramen rotundum laterally.

The lowest portion of the wing is marked by a blunt angle, lying below and a little medial to the foramen rotundum, and representing the processus pterygoideus. The innermost extremity of the wing presents a more sharply marked angle, which projects freely inward, where it comes into close contact with the parasphenoid bone, or internal pterygoid plate, the upper extremity of which lies immediately ventro-medial to it. Medially and caudally this angle is separated from the alar process by a well-marked groove,—the deepest part of the circular groove which surrounds the union of the alar process and the lateral portion.

The outer margin of the lateral portion projects farther forward than the medial (fig. 14) so that its ventral face looks inward as well as forward. There is no trace of a lamina pterygoidea, perforated by the internal maxillary artery, such as Voit describes in the skull of the rabbit.

Histologically, modification of the cartilage cells in the upper lateral portion of the wing indicates beginning endochondral ossification; Mall found the first trace of the alisphenoid bone in an embryo of 58 days. Medial to the foramen rotundum the cartilage is thinner and of younger character than that elsewhere in the lateral portion, indicating that this was the part which was, perhaps, latest to form.

Gaupp has pointed out that the brain-case of the mammals has, in the orbitotemporal region, been enlarged by the inclusion of a space which, in the lizards, lies below the primitive side-wall of this region, and to which he has given the name 'cavum epiptericum.' This space is the ventral continuation of the cavity which has been described by Voit in *lepus* as the *cavum supraeochleare*. Voit has found in an early rabbit embryo

evidences of the medial attachment of the primitive side-wall of the orbitotemporal region in the form of three rudimentary cartilages, and membrane connecting them, and stretching out from them. From behind forward he finds, first, Restknorpel *a*, surmounting the commissura suprafacialis (continuous with this on one side but not on the other), next Restknorpel *b*, overlying the semilunar ganglion, and connected by a sheet of connective tissue, which overlies the abducens nerve, with the pillar of the dorsum sellae. Restknorpel *b* is quite free. A third rudimentary cartilage, Restknorpel *c*, appears only on the left side of Voit's youngest rabbit skull, and this fact appears to point to its transient nature. It presents itself at about the site apparently of the middle clinoid process, and is attached directly to the side of the Balkenplatte. Between Restknorpeln *b* and *c* the boundary between the primitive cranial cavity and the cavum epiptericum is not clearly marked. From these three Restknorpeln, and from the cartilage intervening, anchorage is afforded for a stout sheet of connective tissue which stretches outward and upward to find its cranial attachment in the lower edge of the taenia marginalis, or, as Voit calls it in this region, the commissura orbitoparietalis. Underlying this membrane, which Voit thinks is, to some extent, the precursor of the dura mater of this region, are several important structures which are primarily outside of the primitive brain case, as in the lizards, but are later taken into the brain case of the mammals; among these have already been mentioned the semilunar ganglion and part of its nerve trunks, part of the facial nerve, the geniculate ganglion and part of the great superficial petrosal nerve. In addition might be mentioned the nerves to the eye muscles, and the internal carotid artery—indeed all the structures in the cavernous sinus. The carotid artery is shown in the youngest stage of Voit winding around the caudo-ventral surface of Restknorpel *c* to enter the primitive cranium, this point marking its original inlet.

From the researches of Voit it would appear that the new floor and side-wall of the cavum epiptericum and the cavum supracochleare are formed by the upper part of the ala temporalis.

the tegmen tympani and its forward extension, and the commissura aliochlearis, together with the osseous elements formed by the parietal, and squamous portion of the temporal, bones. These completely shut off this space, except for the foramina.

In my embryo there do not appear to be any of the nodules corresponding to the three Restknorpeln of Voit. The space corresponding to the above cavities is filled with loose connective tissue containing vessels and nerves. The dura has not condensed, is loose, and spreads outward and upward from the level of the upper part of the pituitary body. Thus the primitive cranial floor and side-wall of this region is not represented in my model. There is, however, what may be a rudiment of the cartilaginous secondary cranial floor—the floor of the cavum epiptericum, viz., the small nodule which I have called the cartilago supracochleare. Voit considers the aliochlearis a part of this floor, which dorsally is directly continuous with the planum supracochleare; this being so it follows that, if the cartilago supracochleare be regarded as a rudiment of the commissura aliochlearis it is then a rudiment of the cartilaginous floor of the cavum epiptericum, or, more accurately, the cavum supracochleare.

Regio ethmoidalis

The ethmoidal region is the most ventral of the primary divisions of the chondrocranium. With the orbitotemporal region it is directly continuous at three points, as we have seen; medially the septum nasi passes directly backward into the septum interorbitale, and laterally the upper portion of the lateral wall of the ethmoid is united, on each side, with the sphenothmoidal cartilage through the sphenothmoidal commissure and the line of bridges of cartilage dorsal to the latter.

Architecturally considered the principal elements entering into the construction of the ethmoidal region are those going to form the nasal capsule, but this region also includes certain accessory cartilages, of which there are, in relation to the septum the anterior and superior paraseptals; in relation to the lateral wall (intracapsular) the cartilago meatus medii, and (extracapsular) the paraethmoidal and paranasal cartilages.

The nasal capsule resembles, roughly, a tent, partitioned into equal, paired, lateral rooms or cavities by the septum, or mesethmoid, which lies in the median sagittal plane of the skull. The highest point, or peak, is marked by the crista galli. The roof and sides are formed by the tectum nasi and paries nasi respectively, the posterior or subcerebral portion of the former being broken by the long, paired, irregularly-contoured fenestrae cribrosae (fig. 1), destined to become the cribriform plate. This portion of the superior surface of the capsule takes part in the formation of the median part of the anterior cranial fossa, and represents the anlage of the upper surface of the ethmoid bone.

The incomplete floor (fig. 2), or solum nasi, is formed laterally by the inwardly-turned lower edges of the side-walls, and medially by the lower border of the septum and the anterior paraseptal cartilages. Between the side-wall and the septum is the gaping and elongated basal fissure, which extends ventrally into the incisura narina and dorsally into the *cupuloseptal fissure*, the latter being a very narrow space between the dorsalmost extremities of the nasal septum and the nasal wall, almost completely filled by perichondrium. The floor is almost entirely covered in by membrane bones—the maxilla, the palatine and the vomer—these closing off the inferior nasal meatus below, and marking the upper delimitation of the oral region. The three elements, tectum, paries and solum nasi, combine to form the shell-like structure known as the ectethmoid. Laterally the dorsal portion of this is in relation to the orbit.

The nasal septum or mesethmoid (fig. 11) is a vertical and roughly pentagonal plate of cartilage, constituting the ventral end of the central stem of the chondrocranium. The dorsal border is marked above by the interrupted line of attachment of the dorsal surface of the tectum nasi, which separates the surface of the nasal from that of the interorbital septum; below this these surfaces have no definite delimitations. The cranio-dorsal border, after passing over from that of the interorbital septum, runs horizontally forward for a short distance, and then mounts rapidly and evenly to reach the highest point of the chondrocranium in the conspicuous crista galli. The latter marks the

widest part of the septum. The outer edges of this border, except in the upper portion of the crista galli, show short lateral eminences, which project into the fenestra cribrosa. These projections will later coalesce with others from the upper edge of the side-wall, and thus form the dorsal portion of the cribriform plate. In front the crista galli passes over upon the cranio-ventral border, which is straight, and sharply inclined downward, while laterally it is directly continuous throughout with the ventral portion of the tectum nasi. By reason of the fact that the tectum rises somewhat, as it springs from the septum, the upper border of the latter lies here at the bottom of a shallow furrow, the sulcus suprasedalis (fig. 1), which reaches from the crista to the ventral tip of the capsule.

The ventral border of the septum is straight and free, and marks the anterior extremity of the mesethmoid, forming the medial limit of the incisura narina, the representative of the fenestra narina of some of the lower forms. At its lower end it meets the caudal border at an angle of 113° . The caudal border is almost straight, horizontal, and thickened throughout, but much more so dorsally than ventrally, so that it resembles a cone, this similarity being rendered more striking by the fact that the transition to the thin part of the septum above is quite abrupt, thus resulting in the formation, on each side, of a shallow furrow (figs. 14 to 18). Near the ventral extremity the caudal border shows on the right side, but not on the left, a lateral connection with the cartilages of Jacobson (fig. 11), and, in front of this, bilateral unions with the ventro-lateral processes (Fawcett '11), the latter appearing immediately behind the front end. Projecting backward from the posterior of these attachments, lying parallel with and below the caudal border, in a position corresponding to about the middle of its ventral half, may be seen the cartilages of Jacobson, or the anterior paraseptal cartilages (figs. 2 and 18). Behind these the dorsal portion of the border, in its caudo-lateral aspect, is covered by the thin plates of the vomer (fig. 2).

The surfaces of the septum are for the most part smooth, but in the region below the crista galli there is a deflection to the left;

on the right side this appears as a well-marked furrow, running from above downward and forward (fig. 11). In the area just above the anterior paraseptal cartilage the furrow lying above the lower, thickened border is somewhat deepened, and in this hollow is to be found the anlage of the organ of Jacobson (figs. 11 and 18).

An interesting feature of the mesethmoid is found about the middle of its dorsal half (the part corresponding to the future lamina perpendicularis of the ethmoid bone), in the form of two paired, elongated cartilages, very small in size, which lie parallel with, and close to, the surface. These may be termed the *superior paraseptal cartilages* (figs. 11 and 16), and though mature, their cartilage is younger in type than that composing the adjacent septum. The cartilage on the right is somewhat the longer, measuring 13 mm., the left being 11 mm., and in direction the right is parallel with the caudal border of the septum, while the left runs slightly upward, as well as backward. The nasal septum, opposite the anterior part of each cartilage, and for a short distance in front of it, shows a slight swelling, and the anterior part of the paraseptal cartilage lies just lateral to and somewhat below this. So closely does the paraseptal cartilage lie to the nasal septum that it is difficult to make out a separation, but by the aid of the high power and close examination it is seen that the cartilage is separate from the septum, except at two points on the right side, and one on the left. On the right side the connection points are at the anterior extremity, and about the middle, while on the left the sole union is at the anterior end. Thus each cartilage presents a free, posteriorly projecting extremity. From the caudal edges of each cartilage there stretches downward a sheet of young connective tissue. Figure 16 shows the relationships of the superior paraseptal cartilage to this sheet, and it will be seen that, where the cartilage is free from union with the septum, as it is on each side in this figure, the relationship of the cartilage to the membrane is somewhat similar to that of a sesamoid bone to its tendon, for the real upper connection of the membrane appears to be somewhat above the cartilages, where it becomes continuous with the perichondrium

of the septum, as shown in this figure. The two sheets unite below the septum, and in this region, which marks their thickest part, they contain the slender spicules of membrane bone which represent the vomer. Though they are found throughout the extent of the vomer they disappear shortly beyond its extremities.

Fawcett ('11) in his description of the paraseptal cartilages finds a similar sheet of condensed mesenchyme, and gives to it the name "suspensory membrane," stating that it envelops the anterior and posterior paraseptal cartilages, and extends between them; further that in the interval between these cartilages the vomer is developed (following Zuckerkandl '08). I am unable to discover in my model any trace of this "suspensory membrane" in front of the ventral extremity of the vomer, so that it has, obviously, nothing to do in this stage with the support of the anterior paraseptal cartilages; moreover it is not found behind the dorsal extremity of the vomer, and hence cannot function in the suspension of the processus cupularis posterior (posterior paraseptal cartilage of Fawcett) which evidently represents the last rudiment of the posterior transverse lamina of such forms as the rabbit (Voit). Furthermore, since the cellular composition of this membrane is apparently the same as that which forms the membranous anlage of any of the membrane bones, and its situation is that which will be occupied by the future upward-growing vomer, and since the vomer is to be found within its thickened caudal portion, it would appear that it is simply the membranous anlage of the vomer. The term 'suspensory' would seem to be misapplied, since the bony elements enclosed by it cannot be said to be suspended, any more than the early osseous spicules of any other membrane bone may be said to be suspended in their membranous anlagen, and the cartilaginous elements are not enclosed by it; indeed the so-called posterior paraseptal cartilages, as Fawcett himself states, are continuous with the lateral walls of the nasal capsule (fig. 14). Fawcett evidently believes that this membrane once sustained the cartilago paraseptalis communis, of such forms as the rabbit, and that the vomer is a covering bone which surrounds and takes the place of this

cartilage when it disappears, although the vomer is present along with the cartilago paraseptalis communis in the skull of the rabbit (Voit). It appears evident from a study of my slides that the vomer attains its adult condition by advancing upward in this sheet of mesenchyme, and thus comes to enclose the septum. What part, if any, the superior paraseptal cartilages play in the development of the vomer, or indeed what their real significance is, I am unable to say.

At the ventral end of the caudal border of the mesethmoid, and lying almost parallel with it, there are to be seen, upon either side, the small, straight, rod-like ventro-lateral processes (figs. 2, 3 and 18), 9 mm. in length in the model, connected by their ventral extremities with the septum (fig. 11), but having their dorsal ends free, the greater part of their length being separated from the septum by perichondrium. The condensed mesenchyme of the ventral tip of the maxilla appears immediately beneath them (figs. 2 and 18), and their material is cartilage of the same character as that of the adjacent septum. Though their dorsal extremities come into close contact with the cranio-ventral process of the median Jacobsonian cartilage, they are not connected therewith.

The Jacobsonian or anterior paraseptal cartilages (figs. 2, 3 and 18) consist of two paired masses, medial and lateral, found immediately dorsal to the ventro-lateral processes. The medial mass is a quadrangular, inwardly concave plate, 28 mm. long, whose long axis is parallel with the lower border of the mesethmoid, with which its upper edge is in close apposition, being, for the most part, only separated by perichondrium. It is much the larger of the two, and lies at a lower level than the lateral mass. The ventral extremity of the plate is drawn out to a rather sharp free point, known as the *ventral process* (fig. 2), its tip lying just below the septum. The lower border is marked, rather nearer the ventral than the dorsal extremity, by a projection, directed downward, which, however, is terminated by a sharp, backwardly turned point of cartilage, this structure being known as the *caudal process* (figs. 2 and 18). The dorsal termination is very blunt: it may be described as the dorsal bor-

der. The cranial border, almost parallel with the caudal border of the mesethmoid, presents near the ventral process a long, slender limb 1 cm. in length, which curves upward and forward, to come into close contact with the dorsal tip of the ventrolateral process, and thus to reach the most ventral extremity of this cartilaginous mass. On the left side this off-shoot, which may be known as the *cranio-ventral process* (figs. 2 and 18), is disconnected from the septum, though only separated therefrom by a thin sheet of perichondrium, but on the right side a connection to the septum appears near the ventral end of the process. This connection, however, is very meager, the surrounding perichondrium almost cutting it off, and it would seem to be secondary. The cartilage of which the plate is composed is similar to that of the septum within. The two plates enclose a space, open below, which is filled with dense connective tissue, and at the dorsal border of the cartilages the ventral tips of the vomer are seen in it. The ventral process is connected laterally with the lateral Jacobsonian cartilage by cells of procartilage.

The lateral member of the anterior paraseptal cartilages (figs. 2-3) is a short, curved rod, 1 cm. in length, whose concavity is directed downward, forward and slightly inward, lying almost parallel with the cranio-ventral process of the medial mass which is to be found immediately internal to it. The entire lateral mass is of a younger type of cartilage than that composing the larger Jacobsonian cartilage, and upon examining the sections it is seen that it is connected with the latter at a point near the ventral process by procartilage. Otherwise the lateral mass is quite free. The adjacent portion of the lateral wall is marked by the prominent paraseptal process (fig. 2), whose tip is composed of young cartilage, and between this and the lateral Jacobsonian cartilage there is a zone of loose tissue which suggests an earlier connection between these points such as exists in the rabbit (Voit) in the form of the anterior transverse lamina. The ectethmoid would then be united with the medial Jacobsonian cartilage, the lateral member being probably a rudiment of this lamina.

In the ectethmoid (fig. 12), as we have seen, there may be recognized a roof, or tectum nasi, a lateral wall, or paries nasi, and a floor, or solum nasi. Its only connections are made medially with the septum (figs. 11 and 12) and laterally with the cartilago sphenothmoidalis (fig. 1), both unions being through the roof. The posterior, or subcerebral portion of the tectum nasi is as yet imperfectly developed, and is concerned principally in the formation of the lateral delimitation of the fenestra cribrosa, the representative of the future cribriform plate. Dorsally this subcerebral portion is wide and flattened, and forms part of the ventral boundary of the fissura orbitonasalis. Medially it is connected with the nasal septum by a short line of attachment, interrupted by small foramina (fig. 12) while laterally it is bounded by the broken line of union with the cartilago sphenothmoidalis. This portion narrows as it is followed ventrally, and forms the rather uneven lateral boundary of the fenestra cribrosa.

The ventral border of this fenestra is formed by the dorsal edge of the prominentia superior (fig. 1), which is a medial continuation of the sphenothmoidal commissure connecting this to the nasal septum in front of the crista galli. Projecting backward into the fenestra cribrosa from the point of union of the sphenothmoidal commissure with the superior prominence is a short spicule of cartilage, known as the *processus cribroethmoidalis* (fig. 1), also present in the model of Hertwig. It forms the median boundary of a small incisure, known as the *incisura cribroethmoidalis* (fig. 1) and appears to be the representative of the foramen cribroethmoidale of the rabbit (Voit), through which the anterior ethmoidal branch of the ophthalmic division of the 5th nerve passes into the nasal from the cranial cavity, to emerge, as we shall see, through the foramen epiphaniale as the external nasal ramus. In the model of Hertwig the incisure is still unclosed dorsally. It represents the ethmoidal fissure of the mature bone.

The ventral, or precerebral, portion of the roof is attached, throughout its entire extent, to the nasal septum (fig. 12). It

is almost straight, in the sagittal plane, there being a slight depression between the superior and the supraconchal (Sakterwulst—Voit) prominences. In the coronal plane the tectum is convex upwards, and, when regarded from above, it is seen to be widened by the afore-mentioned prominences.

The paries nasi may be divided into a smooth dorsal portion, the planum antorbitale (fig. 3), which forms the ventro-medial wall of the orbit, and a ventral portion, which presents a much more uneven surface, the two grading into one another in the region of the lacrimal duct. Mead states that "in the reptiles a line joining the corresponding place (commissura sphenothmoidalis) with the processus maxillaris posterior would separate the paries nasi from the planum antorbitale. The same is true also of the mammals, although here the planum antorbitale is usually oblique instead of transverse." Upon comparison with such forms as the pig the paries nasi (sensu stricto) of man, comprising the part ventral to this line, is very rudimentary. Dorsally the planum antorbitale terminates in a rather sharp point, and upon examining the inner surface of the ectethmoid it is seen that this tip is turned inward and forward, the cartilage being directly continuous, to form the *processus cupularis posterior* (figs. 12 and 14). The border above this extends upward to the bridge of cartilage joining the dorsal portion of the tectum with the septum; it presents near its cranial extremity a small notch (fig. 12), and below this it is fitted closely to the contour of the septum within, the narrow space between being known as the cupulo-septal fissure (figs. 2 and 14) completely filled with connective tissue. This fissure marks off, upon the septum, the delimitation between its interorbital and nasal portions. The upper portion of the planum antorbitale is in close relationship to the ventral root of the ala orbitalis on account of the very rudimentary condition of the interorbital septum in the mammals. A theory accounting for the shortening of the latter is given by Mead who states: "In the evolution into the nasal capsule of the mammals the posterior part of the capsule of the reptiles has been expanded by the backward rotation of the posterior wall (reptilian planum antorbitale), the pivot being the more solid lateral side."

Below the dorsal extremity of the planum antorbitale is a wide notch, the *dorsal palatine notch* (fig. 12), partly filled by the developing upper portion of the palate bone; below this is a rounded angle, from which the sharply-marked lower border runs forward and outward, with a slight concavity downward, to end, by an upward and inward bend, upon the posterior prominence (fig. 3). When the capsule is looked at from below it is seen that the ventral two-thirds of this lowermost border is concerned with the attachment of the solum nasi (fig. 2). Above, the line of attachment of the sphenothmoidal cartilage marks off the upper surface of the paries nasi from the tectum, and just beneath this line, and close to the surface of the planum antorbitale, but separated therefrom by connective tissue, there is to be seen a small nodule of cartilage, the cartilago paraethmoidalis (figs. 3 and 16) apparently of the same age as that of the adjacent wall. It is oval in shape and bears no apparent important relationship to the neighboring structures. Within the capsule the superior nasal meatus corresponds to the dorsal area of the planum, while the middle meatus is medial to its anterior portion.

The ventral portion of the paries nasi (fig. 3) (*paries nasi sensu stricto*), is thrown into a number of eminences, between which lie corresponding hollows. In its dorsal area is to be seen the *prominentia posterior*, a small swelling upon which the posterior maxillary process appears. The prominence does not extend so far laterally as the lower border of the antorbital plane behind and below it; the latter is continued upward and inward, curving over the summit of the prominence to enclose, in the downward concavity thus formed, the posterior maxillary process. The latter is wide and flat, projects ventrally and is cut off in front and above by a sharply marked furrow, being unbounded below and behind (fig. 17). The slender lacrimal bone lies along its upper border, and a short distance lateral and below is an elongated nodule of cartilage, the anlage of the *processus paranasalis*, which may be known as the *cartilago paranasalis*. This presents a free ventral extremity and a dorsal extremity closely applied to, but not continuous with, the lower aspect of the posterior prominence. It is separated from the

underlying cartilage by a thin sheet of perichondrium, and between it and the processus maxillaris posterior the anlage of the lacrimal duct may be seen. The lower surface of the prominence is rounded, and thus contrasts sharply with the lower border of the planum antorbitale immediately dorsal to it, which is sharply marked, and projects outward and downward beyond the prominence.

The cranial area of the ventral surface presents a smaller eminence, the prominentia superior (figs. 1, 3 and 12), to whose dorso-lateral edge the sphenoethmoidal commissure is attached and which medially goes over into the septum. It is to be found just ventral to the fenestra cribrosa, and the small foramen epiphaniale (fig. 3) is to be seen piercing its ventro-lateral edge. It represents a small cavity, found in the inner capsular wall. This prominence is separate from a larger one, situated upon the lateral wall, some distance in front and below, which I regard as the structure called by Voit the Sakterwulst in the skull of the rabbit. Between these prominences a shallow furrow may be observed, and this opens below into a prominent pit, delimited by the Sakterwulst, and the superior and posterior prominences. This pit corresponds, I believe, to the sulcus lateralis anterior which Voit describes in the skull of lepus, and it follows that the region dorsal to it must represent the wall of the recessus lateralis. The latter is, however, very rudimentary in man, and the corresponding area of the lateral wall in my model appears to be collapsed, when compared with the condition in the rabbit as shown by Voit's illustrations. The anterior prominence of Voit is not to be seen.

The Sakterwulst (supraconchal prominence) is the most conspicuous of all the prominences in the human ectethmoid at this stage, and presents a sharply-marked summit. Behind the latter may be seen an undulating ridge, which passes successively backward, across the sulcus lateralis anterior, over the ridge of the posterior prominence, to reach the lower border of the planum antorbitale (fig. 3). The Sakterwulst corresponds to the ventral extremity of the middle meatus of the nose; below it is a groove leading downward and backward to an incisure in the lower

border of the ectethmoid, the incisura post-transversalis (figs. 3 and 12), behind the processus paraseptalis, the latter being the representative of the anterior transverse lamina of the rabbit (Voit). The ventral surface of the Sakterwulst slopes downward, forward and inward to the upper part of the incisura narina. Upon the edge of this there is, at this point, a small projection, the representative of the cartilago cupularis of the lower forms (fig. 12).

The ventro-caudal portion of the paries nasi is raised into a slight eminence, and upon the ventral edge of this, which bounds the incisura narina, the processus alaris superior appears (figs. 2 and 12) this being, however, but rudimentary in man when compared with such forms as the rabbit. The most prominent feature of the lower edge is the elongated and slender paraseptal process, which points inward, backward and downward.

The solum nasi is formed mainly by the inferior nasal concha, which appears as the inturned lower edge of the paries nasi, and in part by the rudiments of the laminae transversales anterior and posterior, represented respectively by the processus paraseptalis and the processus cupularis posterior. The dorsal portion of the inferior concha is corrugated, and presents a free posterior edge, which meets its median border at a right angle. This dorsal edge is inwardly continuous with the lower extremity of the middle concha (fig. 12), where it joins the inner aspect of the planum antorbitale; with the latter it forms a notch, lodging the ventral portion of the upper border of the palate bone, and known as the *ventral palatine notch* (figs. 2 and 12). A rounded ridge, directed backward and inward, appears upon the lower surface, and separates two grooves, the medial being the more well-marked. This ridge is represented in the floor of the middle meatus by a wide groove which runs backward and inward to terminate by passing over the dorsal edge of the inferior concha, which thus shows a marked concavity upward.

The ventral portion of the solum is narrow and more sloping than the dorsal, and is also smoother. It terminates ventrally at the post-transverse incisure. The medial border of the inferior concha is thickened except at its extremities, and forms the lateral boundary of the basal fissure (fig. 12).

The medial surface of the ectethmoid is complicated, but is, in a general way, concave. Dorsally may be seen (figs. 12 and 14) the processus cupularis posterior projecting forward freely into the mesenchyme. This is the last remnant of the lamina transversalis posterior which is found in the rabbit (Voit), in which animal it connects ventrally with the paraseptal cartilage, but in my model there is no such connection. This is the only part of the dorsal border which turns inward, and is thus the sole representative of the median part of the cupola posterior, described for many of the lower forms. A portion of the dorsal border, a little below the lower margin of the bridge of cartilage which joins the tectum nasi to the septum is partially cut off by a thin sheet of perichondrium, and it also presents a slight thickening. Its cartilage at the ventro-cranial edge is slightly younger than that found in the adjacent wall. This may be the homologue of one of the ethmoidal conchae of the lower forms (fig. 12). Below the dorsal portion of the tectum is to be seen a small fossa, probably the representative of the sphenothmoidal recess of the adult condition, and this is bounded below by the anlage of the superior nasal concha (fig. 16). The latter is a small ridge of cartilage which stretches across from the medial surface of the planum antorbitale behind to the attachment of the middle concha with the wall, in front.

Below and behind the superior concha is to be seen the superior meatus of the nose (fig. 12), and it may be noted that this narrows below by reason of the fact that the line of attachment of the middle concha, which forms its lower and anterior boundary, runs from above downward, as well as backward.

The middle nasal concha (figs. 12 and 16) is somewhat larger than the superior, and presents an irregular lower edge which may be followed from a swelling of the lateral wall (which corresponds to the antero-lateral sulcus of the outer surface) backward and downward to terminate at the apex of the ventral palatine notch by running over upon the dorsal edge of the inferior concha. The edge of the middle concha is composed of very young cartilage. The middle concha is perforated, near its upper end, by a small foramen, filled by connective tissue. Its

medial surface, in its ventral and upper portion, looks inward, but as it is followed dorsally it is seen to become directed backward, and also to become much narrower, so that the dorso-caudal extremity even looks slightly outward, being separated from the adjacent medial wall of the planum antorbitale by a perceptible interval, the lower limit of the superior meatus, while its dorsal edge shows a slight prominence projecting backward a short distance before the lower end is reached.

The middle meatus (figs. 12 and 16) is quite capacious—almost cavernous—in appearance. Above, it is roofed in by the overhanging middle concha, and below it is delimited by the shelf-like inferior concha, these two conchae meeting dorsally to bound its dorsal extremity, this latter being a deep recess. Ventrally the deepest portion of the meatus is marked by a furrow, which corresponds to the *Sakterwulst* on the lateral surface. At approximately the center of the middle meatus, close to the lateral wall, but separated from it by a layer of connective tissue, is a small nodule of very young cartilage, surrounded, by a shell of procartilage, the latter connecting the nodule dorsally with the wall of the ectethmoid, which shows a prominence at this point. This nodule, which is known as the *cartilago meatus medii*, is situated at a point of the lateral wall just opposite to the posterior maxillary process of the lateral surface (fig. 17), and is surrounded by loose connective tissue. It occurs on both sides, but what its significance may be is uncertain.

At the root of the paraseptal process there may be seen a small hollow, corresponding to the eminence upon the outer surface. The under surface of the ventral portion of the tectum nasi is smooth, concave, and presents, laterally and above, the foramen epiphaniale, aforementioned (fig. 12).

VISCERAL ARCHES

Only the upper two visceral arches, representing Meckel's cartilage with the auditory ossicles and Reichert's cartilage, are shown in the model.

Meckel's cartilages (figs. 3-4) comprise two irregularly curved rods, each of which passes from the ventro-lateral recess of

the otic region to the site of the future mandibular symphysis, and form by the approximation of their ventral, upturned extremities the apex of an angle, the sides of which enclose the triangular area occupied by the structures composing the floor of the mouth. In cross-section each rod is seen to be of the mature type of cartilage and shows an almost uniform diameter, although the middle of the shaft is characterized by a slight but elongated spindle-shaped thickening, while the ventral upturned extremity is thickened and flattened ventro-dorsally. The dorsal extremity of the shaft, too, just before it becomes continuous with the malleus, shows a short fusiform expansion.

After leaving the ventro-lateral recess of the otic capsule, where it is directly continuous with the malleus, the cartilage proceeds forward, inward and downward, lying quite close to the pars cochlearis of the otic capsule (4 mm. in the model). It then changes its direction, passing almost directly forward, and only slightly downward and inward, thus forming, in the portion below the ala temporalis of the sphenoidal anlage, a wide curve with its concavity directed outward, forward and upward. This direction is maintained until the cartilage reaches a point a short distance in front of its termination, when it turns rather abruptly upward, and its tips become flattened, their medial edges being approximated in the midline, but being separated by a thin sheet of connective tissue, as is usual in homo. Thus a second curve, with its concavity upward and slightly outward, is formed. Upturning, enlargement and flattening of the anterior end is noted by Low first in the 18 mm. stage, and is persistent, the tip becoming later constricted off, and appearing in the 95 mm. stage of Low as a small nodule above the symphysis.

Excepting the small portion made up of the posterior fusiform expansion the shaft is flanked laterally by the covering membrane bone of the mandible, which also overlaps the ventral extremity in the manner shown in the Hertwig model, and which was noted by Low as early as the 18 mm. stage. The terminal upturned portion of the cartilage shows a general enlargement of the cells, and to the lateral surface of this the covering bone is applied very closely—indeed it would appear that this area is encased by a thin plate of perichondral bone. Within this the

cells are still larger and vacuolated, and where it fuses with the mandibular bone there is what appears to be a beginning center of endochondral ossification. Here the ground substance is much more deeply staining, and at one point a bud of osteoblastic tissue appears to be invading the cartilaginous mass. Elsewhere, however, the cartilage and covering bone are separated by a narrow interval, containing connective tissue, and nowhere else in the cartilage are there any indications of ossification.

Just beneath the posterior expansion of the shaft is seen the small, flattened tympanic bone (fig. 2), while medial and above the latter, lying with its long axis parallel to that of the Meckelian cartilage, and immediately applied to it, is the slender goniale. Above and lateral to the posterior extremity of the shaft is to be seen the squamo-temporalis.

Ventrally the mylohyoid muscle is attached to the perichondrium of Meckel's cartilage, but in the middle third its attachment is to the inner table of the mandible and its dorsal prolongation, this connection being established just above the cartilage. Hence it would appear that the mylohyoid ridge of the mature bone indicates the original position of Meckel's cartilage.

By reference to the Hertwig model it is seen that in the interval between the 40 mm. and 80 mm. stages there has been a modification of the curve of the cartilages, the dorsal curvature having been eliminated in the older stage, so that the proximal portion of the cartilage is forced away from the cochlea and the angle at the symphysis considerably widened. It would appear that in the process of development the cartilage grows more rapidly in length than in thickness, and hence with advancing age it becomes progressively more slender. This is brought out clearly when my model is compared with the earlier Low ('09) models on the one hand and with the Hertwig model on the other, the cross-section of the rod in the latter being less when compared with its length than is the case in my model, and much less than that of the earlier Low models.

Evidence of beginning ossification and resorption of the cartilage opposite the interval between the lateral incisor and canine tooth germs is first noted by Low ('09) in the 31 mm.

stage, in the form of enlargement of the cells, while in the same region, in the 36 mm. stage, he mentions ossification of the perichondrium with vacuolization and enlargement of the cells. Again, in the 55 mm. stage, he figures this region of Meekel's cartilage almost surrounded by bone, and undergoing resorption. Hertwig's model shows a complete investment of covering bone in this region. My model is intermediate between the 36 and 55 mm. stages of Low, the cartilage being only half surrounded by bone, and ossification just commencing within.

Of the auditory ossicles all are represented in the model (figs. 2 and 3). The malleus, as has been stated, is directly continuous ventrally with the shaft of Meekel's cartilage. Dorsally it is cut off from the incus by perichondrium, though in the model the two are represented as continuous, a lateral furrow marking the intervening boundary. The head of the malleus is large and rounded, and lies just lateral to the tegmen tympani, being separated by connective tissue; its manubrium is long, thickened proximally, and its tip is closely applied to the promontory of the cochlea, a condition which is strikingly different from that found in the model of Hertwig, where there is a considerable interval between these parts, caused apparently by the expansion of the middle ear region, which has thrust the upper end of Meekel's cartilage, with its affixed structures, outward. In the angle between the manubrium and Meekel's cartilage the tympanic and goniale are seen. The goniale, which represents the future anterior process, is at present unattached to the malleus, and remains so till the end of the fifth month (Broman '99).

The incus is completely separated from the malleus, in front, and from the otic capsule, behind, by perichondrium, and presents a body to which is attached a long and a short limb, morphologically resembling the adult condition. The incudostapedial articulation is formed of condensed mesenchyme, and marks the apex of a right angle, open upward, outward and backward, formed by the long limb of the incus and the two limbs of the stapes, and in this angle the facial nerve is to be found.

The stapes at this stage is circular in form, the base being imperfectly developed. The limbs are represented by well-defined, round cartilaginous rods.

Reichert's cartilages (figs. 2 and 4) are the paired cartilaginous rods, lying below the posterior half of Meckel's cartilage, which form the posterior part of the hyoid arch. Each is roughly the shape of a walking cane with a curved handle and a somewhat bent shaft. The handle-like portion is found between the lower end of the crista parotica and the lateral extremity of the jugular foramen, the fenestra perilymphatica being above it, and the paracondyloid process below. It lies quite free in the mesenchyme, separated by that tissue from the otic capsule, a condition agreeing with that described by Jacoby for an earlier stage, in which the cartilages were in contact with the otic capsule, but not fused with it, as they are shown to be in the Hertwig model of a later stage. Each cartilage is of almost uniform diameter throughout, and its direction is from behind forward, inward and slightly downward, the shaft presenting a slight outward and downward bowing, where it sweeps around the promontory of the cochlea, and a gradual inturning toward the distal extremity. As it passes forward the shaft gradually separates from the promontory, to which it is closely approximated, without being actually in contact, and at the same time draws closer to Meckel's cartilage. The terminal anterior tip lies just medial to the angle of the mandible (fig. 2), and above the greater cornu of the hyoid cartilage, which it overlaps for a short distance, without actually becoming contiguous, and it shows a rather younger type of cartilage than the rest of the shaft. The latter differs from that of the chondrocranium in being more reddish in stain.

MEMBRANE BONES

All of the purely membrane bones of the skull are represented in my embryo, though they are as yet but imperfectly developed. The interparietal appears, as has been noted, just lateral to the dorsal occipital prominence as a faintly staining spicule of osseous matter, but owing to the lack of the dorsal sections the extent of the bone could not be ascertained, and hence it was not shown in the model. Though this bone does not appear in the model of Hertwig it must have been present, as this embryo was con-

siderably older than mine. According to Mall ('06) the centers for the interparietal (one on each side) appear on the 57th day and unite on the 58th day.

Situated some distance above the otic capsule, and forming a part of the side wall of the cranial cavity, is the network of bone which is the anlage of the future parietal (fig. 4). It is roughly of diamond shape, with the long axis directed cranio-caudally, and medial to its lower border is the disappearing parietal plate, the two layers, cartilage and bone, being separated by a slight interval filled with connective tissue. There is a considerable area separating this bone from the frontal, and it is also widely separated from the interparietal, there being a very large area at the back of the cranium, behind the parietals and above the tectum posterius, uncovered by bone or cartilage.

The parietal bone is not so dense as the frontal, showing that it is probably later in appearing. Its structure presents no definite center, it being a fine reticulum throughout, though, for technical reasons, it has been represented in the illustrations as a solid plate.

The frontal bone (fig. 4) is similar in structure to the parietal, and encloses the lateral and ventral parts of the anterior cranial fossa. In it two main parts may be distinguished, a vertical portion, convex antero-laterally, and a horizontal portion, which is slightly arched upwards for the accommodation of the structures of the orbit; these being the anlagen of the corresponding parts of the adult bone. Joining these portions is a well-marked, rounded ridge, the representative of the future supra-orbital ridge, and here the bone is most densely deposited, especially in the central part of its extent, indicating, probably, that in this position the first masses of osseous tissue were laid down, and not in the region where the frontal eminences will later appear, as is stated in the textbooks. The entire margin of the bone, as well as the greater area of the posterior half, is but a tessellated plate composed of intercrossing osseous spicules. The anterior extremity is rounded, and shows no definite resemblance to the mature condition; it is separated by a considerable interval

from the nasal bone, and almost touches its neighbor of the opposite side, this being the part of the bone which most nearly reaches the median plane. Posteriorly it gradually recedes from this plane, the entire medial edge of the horizontal portion underlying the outward-slanting ventro-lateral edge of the sphenothmoidal cartilage. Its dorsal extremity is separated by a considerable interval from the parietal bone. It also, for technical reasons, is figured as a plate.

The squamosal (figs. 2, 4 and 13) is a somewhat fan-shaped bone, in which two distinct portions may be recognized; a posterior flattened plate, covering laterally the auditory ossicles and the upper part of Meckel's cartilage, and a ventral, elongated spicule of bone, the zygomatic process, whose tip is found just above the dorsal tip of the zygomatic bone. The upper edge of the flattened portion is convex upward, and is somewhat serrated, the lower border being slightly concave, and passing directly into the zygomatic process. The latter, owing to its lying parallel with the sagittal plane, meets the flattened portion at an angle, as the latter looks outward and forward (fig. 2).

The zygomatic bone (figs. 2 and 4) is somewhat quadrilateral in shape, and thus bears a resemblance to its adult condition. It already shows a body, with four angles, three of which terminate in marked projections. The body is flattened and thin, and the ventral part of its medial surface is in close apposition, though not in union, with the zygomatic process of the maxilla. From the dorso-caudal angle there projects backward a spicule of bone, whose dorsal extremity underlies the ventral extremity of the zygomatic process of the squamosal, thus identifying it with the zygomatic process of the zygomatic bone; the zygomatic arch is, accordingly, incomplete. From the cranial angle there projects upward and slightly backward the rather blunt, but strongly marked, frontal process, lying somewhat ventro-lateral to the upper extremity of the ala temporalis, and a slender elongated and inwardly curved infra-orbital process overlies the outer border of the zygomatic process of the maxilla. The caudo-ventral angle of the body is well-marked, and represents the anlage of the future malar tubercle.

The lacrimal (figs. 4 and 17) is a slender spicule of membrane bone lying along the upper and lateral part of the cartilaginous posterior maxillary process, its long axis being directed from behind forward, inward and slightly upward. Its middle part presents a slight thickening, and the naso-lacrimal duct lies immediately lateral to it.

The nasal bone (figs. 1 and 4) is represented in the model as a thin plate, rounded in outline, flattened, and about 1 cm. in diameter, lying upon the part of the upper surface of the tectum nasi which represents the medial surface of the supraconchal prominence. Behind this plate, and lying close to it, are several minute nodules of membrane bone, which also contribute to the formation of the adult bone. The nasal anlagen lies in a mass of dense mesenchyme, which overlies the entire nasal capsule, similarly to the formation described by Fawcett ('10 a and '10 b) in a 30 mm. human embryo.

The tympanic (figs. 2 and 4) is a short, flat plate of membrane bone, lying beneath the posterior extremity of Meckel's cartilage, and in front of the malleus. Its anterior part is slightly wider than the posterior, and its long axis lies parallel with that of the cartilage, its flattened surface being applied to the latter. The bone is found in an isolated anlage of condensed osteogenic mesenchyme, and is placed lateral to and below the goniale. When compared with the Hertwig model it is seen that the only part which is as yet laid down is the anterior widened extremity, there being no evidence of the ring-like form of the later bone.

By the subsequent outward swinging of the ossicles and attached Meckelian cartilages consequent upon the expansion of the region of the middle ear the plane bordering upon Meckel's and Reichert's cartilages is changed in direction, so that its outer surface looks almost directly downward, as shown in the model of Hertwig, instead of outward, as is the case in my model, and at the same time the interval between these two cartilages is relatively narrowed. This interval the tympanic bone comes to occupy, its dorsal half, as yet undeveloped in my embryo, being applied to the cartilage of Reichert in the Hertwig model, the long process of the malleus occupying a position within the ring.

The goniale (figs. 1-2) is a short, somewhat flattened, rod of membrane bone, lying immediately below and medial to the posterior fusiform enlargement of Meckel's cartilage, and so close to the latter that it appears to be developed from the perichondrium covering its surface. Its long axis is parallel with that of Meckel's cartilage, and its anlage is quite separate from that of the tympanic, which lies on a lateral and caudal plane. Its posterior end approaches close to the neck of the malleus, but does not actually become continuous therewith until the end of the fifth month (Broman '99), when it becomes the anterior or Folian process of that ossicle, a fact which was demonstrated by Dreyfuss ('93). Gaupp ('05 and '11) has given strong reasons for the identification of the processus Folianus with the goniale of the reptiles, and it is on his authority that I have used that name for it here. The bone is shown in the Hertwig model, and also appears in Low's illustration from a 95 mm. human embryo under the name of 'processus folianus.'

The vomer (figs. 2, 16, 17 and 18) is represented by two slender strips of bone lying one on either side, immediately caudolateral to the lower border of the mesethmoid, the two almost enclosing this border excepting for a narrow strip between their lower edges. The dorsal extremity of each strip is blunt, but the ventral is drawn out to a fine point, and near the latter there is a bridge between the two bones. The ventral point projects only a short distance in front of the dorsal border of the larger Jacobsonian cartilage.

The maxilla (figs. 2, 4, 16 and 17) is an irregular mass of cancellous bone which lies in a notch on the ventro-lateral aspect of the ectethmoid, and it already shows the frontal, alveolar, palatal and zygomatic processes. The central mass of the bone is irregularly triangular in form upon coronal section (fig. 17), the longest side of the triangle being applied to the ethmoidal cartilage, while the medial angle represents the palatal process, the upper angle the frontal process, and the remaining angle, which is a right angle, the alveolar process. The pointed, upward-directed frontal process reaches a level somewhat above

that of the lacrimal; its posterior surface forms the anterior wall of the space containing the naso-lacrimal duct (fig. 4). The palatal process does not quite reach to the median plane and anteriorly it is separated by a notch from the extremity of the alveolar process: its medial border is below and lateral to the vomer, and its posterior border underlies the palatine bone for a considerable distance.

The alveolar process is a very irregular ridge of bone, occupying the inferior and lateral edge of the maxilla, and its anterior end extends medially almost to the mid-line, coming to lie in advance of the palatine process. In its lower aspect may be seen the depressions for the future dental alveoli. The zygomatic process is a more lateral coarse spicule of bone, which projects backward and outward until its extremity almost touches the inner surface of the zygomatic bone. It is perforated from above downwards by foramina, and just before it joins the body of the bone it sends up a small process, which marks off a groove occupied by the second branch of the trigeminal nerve (fig. 17). There is as yet, therefore, no foramen for this nerve trunk. The posterior edge of this process is continuous inwardly with the posterior edge of the palatal process. Anteriorly a spicule from the upper part of the anterior extremity of the alveolar process projects forwards, and lies lateral to and below the anterior part of the anterior paraseptal cartilage, to which it is closely applied. This is the anlage of the anterior nasal spine.

The palate bone (figs. 2, 3, 14, 15 and 16) is a thin, delicate lamella of osseous tissue, strongly concave inward, which walls in the dorsal portion of the nasal cavity. Its borders are serrated; the upper being fitted into the dorsal palatine notch of the ectethmoid, and ventrally to this into the ventral palatine notch. The incurled lower edge (fig. 2) which becomes the palatal plate, approaches but does not meet its fellow of the opposite side, being separated from it in the model by a space of 15 mm. Dorsally the anlage of the pyramidal process is represented by a thickened and irregular projection which lies ventral to the medial pterygoid lamina.

The medial pterygoid plate (figs. 2, 3 and 10) is represented by an elongated and somewhat spiral rod, lying behind the palate bone and medial to the lateral portion of the ala temporalis. Two distinct portions may be recognized in it, the upper part being osseous and the lower cartilaginous. The former is bent dorsally and from its most posterior extremity, which is about 3 mm. from the medial angle of the lateral portion of the ala temporalis, a short but stout process projects cranio-medially, marking the highest part of the rod. Below it terminates in the cartilaginous hamular process, which points almost straight downward.

The membrane bone of the upper portion is typical in structure, and when followed downward shows a gradual transition into the cartilage of the hamular process. The cells of the membrane bone enlarge, their capsules swell, and at the same time the ground substance becomes lighter in color. Nearer the cartilage which forms a cap to the extremity of the osseous rod the capsules become smaller, the ground substance increased in amount, and the line of transition is not sharply marked. Though the tip of the cartilage partakes of many of the properties of normal cartilage—staining lightly, having a homogeneous matrix, the nuclei surrounded by capsules, and the whole being enveloped by a closely-fitting, sharply defined, thin sheet of perichondrium—yet there are several points of difference which mark out this mass of cartilage as different from that found elsewhere in the primitive skull. The matrix stains slightly more darkly, the nuclei are larger and lighter in color, and the capsules surrounding them are relatively smaller, when compared with the size of the nuclei. Altogether the cells resemble those of membrane bone more than they do those of typical cartilage.

The mandible (figs. 3-4) is a plate of membrane bone lying immediately lateral to Meekel's cartilage, and separated from this throughout its extent by connective tissue, except in a small area in the region of the lateral incisor and canine tooth germs, where, as has been noted, the bone is directly applied to the cartilage, appearing like ossified perichondrium. The posterior part of the bone is wider than the anterior, and is marked

cranially by a distinct indentation, the representative of the future sigmoid notch, which separates the anlagen of the condylar and coronoid processes. Just external to the latter is the zygomatic bone, and internal to it is the cartilaginous pars temporalis of the sphenoid. The lateral surface of the plate shows, about the junction of the anterior and middle thirds, a distinct opening, the mental foramen, and at this point there is a bend in the bone, the direction of the long axis changing from downward and forward to upward, inward and forward. A short distance above the lower border of the bone, and parallel with it, a groove may be seen upon the lateral surface, more distinct posteriorly than anteriorly. This is also shown in the illustrations of Low's older specimens, and appears to represent the line of insertion of the masseter.

The anterior half of the bone shows an inner table, regarded by some authors as the splenial element, which separates the vessels and nerves from Meckel's cartilage, becoming continuous anteriorly with the ossified perichondrium immediately surrounding the cartilage. About the center of the long axis of the bone the so-called splenial element dwindles in height, and terminates as a thin, backwardly projecting spindle of bone, to which the mylohyoid muscle is attached. Anteriorly the membrane bone overlaps the termination of the cartilage of Meckel, and thus shows the condition observed by Low as early as the 18 mm. stage.

On the whole, the shape of the mandible suggests the adult condition, though it is considerably more slender, and the angle is more obtuse. Immediately caudo-medial to the latter is the tip of Reichert's cartilage. There are no accessory cartilaginous nuclei observable, as Low describes in an older model.

Low ('05) has held that each half of the mandible is laid down as a single skeletal element, the dentary, the so-called splenial element being simply an extension of this. The condition in my embryo supports this finding, the main portion of the bone being formed of the dentary, the splenial element being but a thin lamella of osseous tissue, directly continuous below with the

anterior portion of the dentary, and posteriorly terminating in a slender process, as described. Within this medial plate the tooth gutter is well-marked. The condition is very similar to that shown by Low ('09) in his text figure 4.

CONCLUSION

From the foregoing brief account of the morphology of the human fetal skull of 40 mm. it will be seen that it presents, at this stage, several unique features. The structure of the parts surrounding the foramen magnum is significant in casting, perhaps, some further light upon the genesis of this interesting region. The presence of a fairly clearly defined neural arch of the occipital vertebra has been dwelt upon, and its interpretation discussed. The finding of a small nodule of cartilage above and in front of the cochlea is, so far as I am aware, a contribution to our knowledge of the parts in this area. Other new features are the cartilages paraethmoidalis, meatus medii and paraseptalis superior, all of which have been briefly described; in addition may be mentioned the lateral and dorsal cranial cartilages, and the paranasal cartilage, lying lateral to the lacrimal duct.

Finally, I wish to express to Professor McMurrich my sincere appreciation of his kindness in placing at my disposal the material for this research, and the freedom of the Anatomical Laboratory of the University of Toronto, as well as of his abundant advice and encouragement.

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THE CRITICAL PERIOD IN THE DEVELOPMENT OF THE INTESTINES

C. R. BARDEEN

From the Anatomical Laboratory, University of Wisconsin

ELEVEN FIGURES (FOUR PLATES)

In studying the variations of structure found in any part of the body it is of importance to distinguish the less variable from the more variable features. As a rule, the less variable features are associated with fundamental processes occurring early in ontogenetic development, the more variable features with processes of growth and differentiation occurring later in ontogenetic development. Thus while it is comparatively rare to have variation in the number of the digits, finger prints are specific for each individual.

In the development of the human intestines three fundamental loops are formed. First, the entero-colic which extends forward into the umbilical cord and is supplied by the superior mesenteric artery. Second, the gastro-duodenal, which projects to the right at the base of the entero-colic loop and is supplied chiefly by a branch of the coeliac artery. Third, the left colic which projects to the left at the base of the entero-colic loop and is supplied chiefly by the inferior mesenteric artery.

The entero-colic loop undergoes normally an elaborate development and gives rise to the distal part of the duodenum, the jejunum, ileum, caecum, ascending colon and the right half of the transverse colon. The center of the base of the mesentery of this loop is in the region of the origin of the omphalo-mesenteric or superior mesenteric artery from the aorta. This becomes fixed at an early period opposite the first lumbar vertebra and seldom varies in position. The loop itself shows frequent variations in development, some of a fundamental character, others slight. These will be discussed below.

The gastro-duodenal loop is simple and seldom shows fundamental variations, although individual differences in form in the adult are frequent and well marked. Primitively it is of a simple U form, as may be seen in figures 1, 3 and 5. Such variations as are found in the adult seem to be due in the main to variations in the position of the stomach and to variations in the development of the entero-colic loop (for instance, see figs. 10 and 11).

The left colic loop, although it begins its development considerably later than the other two primitive loops (in fetuses 30-40 mm. long, figs. 3, 4, 5, 6, 7) appears to be nearly as constant in formation. It is usually fixed at an early period in the vicinity of the left side of the left kidney. From it develop the left half of the transverse colon, the splenic flexure, the descending colon and the sigmoid colon. Even in cases in which the entero-colic loop shows marked abnormalities in development one usually finds the upper part of the descending colon in approximately normal position (see for instance, figs. 10 and 11). Aside from extremely teratological cases and cases of complete situs transversus I have found only one case clearly described in which the upper part of the descending colon did not lie in the vicinity of the left kidney. In this case, described by Koch,¹ the colon extended in the mid-dorsal line from the pelvis nearly to the transverse part of the duodenum and then bent toward the right side.

The relative constancy in the position of the upper part of the descending colon is probably due to pressure exerted at an early period by the neighboring intestinal coils against the mesocolon of this portion of the gut (figs. 6 and 7). This part of the mesocolon is thus caused to be fused against the dorsal wall of the abdominal cavity. Proximal and distal to this region of attachment of the colon variations in the development and disposition of the colon are frequent.

Figures 8 and 9 exhibit somewhat extreme variations in the development of the left colic loop above and below the region of early fixation. Above this region the splenic flexure has sent out a loop far to the right in front of the stomach, transverse colon

¹ Die angeborenen ungewöhnlichen Lagen und Gestaltungen des menschlichen Darmes, Deut. Zeitschr. f. Chirurgie, Bd. 50, s. 1, 1898.

and liver. Below this region the colon bends across the mid-dorsal line to the right side of the body before descending into the pelvic cavity.

The entero-colic loop undergoes its early development in part in the umbilical cord, in part in the abdominal cavity. The umbilical part is finally withdrawn (in embryos about 40 mm. long) into the right side of the abdominal cavity and subsequently undergoes considerable shifting before the conditions characteristic of the adult are reached. The more fundamental variation in the development of this loop have to do first, with the intra-umbilical and intra-abdominal development of the loop preceding the withdrawal of the umbilical portion into the abdominal cavity and, second, with the process of withdrawal of the umbilical portion, the ultimate disposition of the various parts of the loop within the abdominal cavity and their subsequent development.

The development of the umbilical part of the loop has been most carefully studied by Mall,² who has described the differentiation of three primary coils of the small intestines within the umbilical cord and their elaboration preceding withdrawal into the abdominal cavity. This development is illustrated in figures 1, 2, 3 and 4 which correspond essentially with figures of corresponding stages illustrated by Mall. There seems to be considerable regularity in the early development of this part of the intestines. Probably the conditions within the umbilical cord are fairly constant and there is little there to interfere with free development.

The active development of the entero-colic loop within the abdominal cavity, on the other hand, seems to take place under greater difficulties and to be subject to greater variation. It does not begin until the development of coils within the umbilical cord is well advanced and is relatively most active at the period immediately preceding and during the return of the umbilical coils into the abdominal cavity.

At this period the colon projects nearly straight forwards in the median plane from near the lower border of the stomach into

² Ueber die Entwicklung des menschlichen Darmes und seiner Lage beim Erwachsenen. His Archiv, 1897.

the umbilical cord (figs. 4, 5, 6, 7). This part of the colon, together with the superior mesenteric artery, which runs nearly parallel with it, and the mesentery which is thickened along the course of the artery, especially near the base, forms a stalk or skeletal support from which the mesentery of the small intestine seems to hang (fig. 7). The part of the mesentery of the free small intestines attached to the part of the umbilical stalk which extends or has extended into the umbilical cord may be looked upon as belonging to coils which have developed within the umbilical cord. The part of the mesentery of the free small intestines attached to the abdominal part of the umbilical stalk may be looked upon as belonging to the coils which have developed within the abdominal cavity.

The beginning of the development of the abdominal coils is marked by the projection of a duodeno-jejunal loop to the left beneath the umbilical stalk. In a 27 mm. embryo (figs. 1, 2) this loop extends to the left of the median plane and at its distal extremity bends ventralwards (fig. 2). In a 35 mm. embryo this duodeno-jejunal loop has begun to give rise to distinct jejunal coils (figs. 3, 4). These coils now develop very rapidly so that in a 40 mm. embryo they approximate in extent the ileac coils in the umbilical cord (figs. 5, 6, 7, coils on left side of umbilical stalk). Just before the jejunal coils begin their most rapid development the bursa omentalis becomes considerably distended (figs. 2, 4).

It is difficult to judge whether this distention is an active one which helps to create more space in the abdominal cavity on the left side, or a passive one due to a sudden increase in the space in the left abdominal cavity. At this period the spinal axis of the embryo in the lumbo-sacral region changes from ventral flexion to dorsal flexion. Whatever factors may be involved in this the result undoubtedly is to offer more space for intestinal development in the abdominal cavity. The steps in this latter process are, first, dilatation of the bursa omentalis; second, rapid development of jejunal coils in the left abdominal cavity and a collapse of the previously swollen bursa omentalis, and, third, the return to the abdominal cavity of the ileac coils from the umbilical cord. The

latter process is under way in the 40 mm. embryo shown in figures 5, 6 and 7.

Normally the jejunal or abdominal coils of the small intestines develop simultaneously with the left colic loop, described above (figs. 3, 4, 5, 6, 7). That this coincident development is not constant is indicated by the numerous cases which have been described, in which, with an approximately normal disposition of the upper part of the descending colon, indicating a fairly normal early development of the colic loop, the proximal part of the small intestines has occupied a markedly abnormal position. In these cases, an excellent example of which is given by Strehl³ and another by Huntington,⁴ the primitive duodeno-jejunal loop appears to be diverted from its usual course of development beneath the umbilical stalk and to have been forced to develop, in so far as development has taken place, on the proximal side of the stalk. In the illustration given by Huntington (fig. 10) the duodeno-jejunal loop appears in about the state of development normally found in embryos 24 to 28 mm. long (figs. 1 and 2). In the illustration given by Broman (fig. 11) the duodeno-jejunal loop appears even less advanced in normal development. When the duodeno-jejunal loop fails to develop to the left beneath the umbilical stalk there appears in general to be a subsequent lack of development of the proximal part of the small intestines. In such cases the intestinal coils are probably in the main coils which have developed in the umbilical cord and then have returned to the abdominal cavity. It is probable that in these cases factors which normally leave room for the development of coils below and to the left of the umbilical stalk become inoperative. Possibly the pressure of the liver is greater than normal. Possibly the lower part of the trunk fails to expand as rapidly as normal.

In most of these cases the umbilical part of the colon fails to reach its usual position when the umbilical coils return to the abdominal cavity. Normally, as is well known, it swings across the ventral side of the loops of the small intestine in the left side

³ Arch. f. klin. Chirurgie, Bd. 87, 1908, cited by Broman; Normale und abnorme Entwicklung des Menschen, fig. 326, p. 380.

⁴ Anatomy of the peritoneum and abdomen, fig. 121.

of the abdominal cavity and comes to lie below the pyloric end of the stomach and the liver in the right hypochondriac region. The entero-colic loop viewed from in front thus undergoes torsion in a direction opposite to the movement of the hands of a watch. In the cases now under discussion either no torsion takes place during the return of the intestines, as in Huntington's case (fig. 10), or, much more rarely, reverse torsion takes place, as in Strehl's case (fig. 11). It is not improbable that the same conditions which make difficult the normal development of the jejunal coils from the duodeno-jejunal loop also make difficult the normal return to the abdomen of the coils developed from the umbilical loop.

We may, however, have an apparently normal development of the left colic loop and of the duodeno-jejunal loop and, nevertheless, have a return of the umbilical part of the intestines into the abdominal cavity without torsion. In a case of this kind, illustrated by Jaboulay,⁵ the colic loop and the abdominal coils of the jujunum appear to be essentially normal while the umbilical portion of the colon lies in the mid abdominal region with the caecum in front of the bladder and the umbilical coils of the small intestine (ileum) occupy the right side of the abdominal cavity.

Normally, after the caecum has passed from the umbilical cord into the right hypochondriac region, the proximal portion of the colon gradually becomes lengthened and bent toward the right iliac fossa and the caecum is thus brought to its adult position. Meanwhile a further rotation of the proximal part of the colon and of the ileum takes place so that the ileac coils come to life in front of the caecum and the ileum enters the caecum from the left side. The colic portion of the mesentery of the entero-colic loop becomes fused to the dorsal abdominal wall beginning where the superior mesenteric artery crosses the duodenum and extending toward the caecum. To the left of where the superior mesenteric artery crosses the duodenum the mesentery is fused to the ventral surface of the duodenum. At the left inferior margin of the area of fusion of the colic mesentery we have the line of origin of the free mesentery of the small intestines. The portion of the small

⁵ Province med., 1891, cited by Fredet in Poirier and Charpy, *Traite d'anatomie humaine*, T. 4, fig. 472.

intestines attached through its mesentery to the duodenum we may look upon as belonging in the main to the primitive duodeno-jejunal loop of the small intestines; that portion attached to the left and below where the superior mesenteric artery crosses the duodenum we may look upon as belonging in the main to the umbilical coils of the intestines. The primitive relations of the mesentery of these two portions of the small intestines is shown in figure 7. They undoubtedly vary, to some extent inversely, in development in different bodies. The duodeno-jejunal coils correspond in the main with the transverse coils in the adult, described by Henke, the umbilical coils to the vertical coils.

Exceptionally good illustrations of the attachment of the mesentery of the jejunum and ileum have been furnished by Stopnitzki.⁶

He has pictured 'high forms' running nearly horizontally to the right from the base of the superior mesenteric artery; low forms bowing well to the left of the usual line of attachment and various intermediate forms. He shows the cut base of the mesentery to be widest in the region of the main trunk of the superior mesenteric artery and to taper off from here toward the colic extremity of the ileum and the jejunal end of the duodenum. He has divided the base of the mesentery into twenty parts and the intestinal margin into twenty parts and compared the distance between the corresponding segments. He found that, as a rule, the mesentery showed two regions of maximum width, one near the seventh to eighth and one near the sixteenth to seventeenth of the twenty segments, counting from the duodenal end. In only two out of every twenty-five cases was there a marked departure. In these two cases the mesentery was widest about the middle of the distance between the two extremities of the small intestine. To what extent the proximal and distal halves of the free small intestine, each with its mesentery longest toward the junction of the distal with the middle third, correspond with the parts of the intestine the coils of which originated respectively within the umbilical cord and within the abdominal cavity we have at present no data.

⁶ Untersuchungen zur Anatomie des menschlichen Darmes, Internat. Monatsschr. f. Anatomie u. Physiologie, Bd. 15, p. 219, 1898.

The distribution of the branches of the superior mesenteric artery are, however,*suggestive.

The branches of the superior mesenteric artery which supply the free small intestines may be divided into two sets, those which arise near and distal to the ileo-colic artery and those which arise proximal to this artery. The former branches in the adult, in the specimens I have examined, supply the distal two-thirds to three-fifths of the free small intestines, the former the proximal third to two-fifths. I have found the same thing true in a fetus 22.5 cm. long. The territory supplied by the distal branches corresponds, therefore, very well with the ileum, that supplied by the proximal branches, with the jejunum. The relations of the two sets of branches to the mesentery and duodenum suggests that the former supply the umbilical coils of the intestine, the latter abdominal coils, but a definite proof of this is possible only with injected specimens of early fetuses and these we unfortunately lack. There is, however, good reason to believe that the primitive umbilical coils correspond essentially with the ileum, the abdominal coils with the jejunum. If we take coils 2 and 3 of Mall to represent the jejunum and coils 4, 5 and 6, the ileum we find from the data given by Mall (table 1, footnotes) that the ileum is about four times as long as the jejunum during the period immediately preceding the return of the umbilical loop. As the return of this loop takes place the abdominal coils (jejunum) grow rapidly so that in the 40 mm. fetus studied by me the ratio of ileum to jejunum is as 4:3. After the return of the umbilical coils the ileum grows relatively much faster according to Mall's data so that in a fetus 130 mm. long it is over three times as long as the jejunum. In the latter part of fetal life the adult proportions appear to be reached.

That we are justified in assuming a primitive development of a considerable part of the small intestines within the abdominal cavity may, I think, be deduced from the following table showing the relative development in length of the small and large intestines as compared with the growth in length of the axial part of the body. The data are based chiefly on those furnished by Mall ('97).

From table 1 it may be seen that in embryos between 3 and 17 mm. in length the small intestines grow at about the same rate as the axis of the body while the large intestines grow more slowly.

TABLE 1

To show the growth in length of the intestines¹ as compared with the growth in length of the trunk²

DESIGNATION OF SPECIMENS	LENGTH IN MM.			PROPORTIONAL LENGTH AT SUCCESSIVE STAGES		
	Specimens ²	Small intestine	Colon	Specimens	Small intestine	Colon
II ³	3	1.7	1.5			
IX	17	9.1	3.7	1:5.67	1:5.35	1:2.47
X ³	24	19.0	7.0	1:1.41	1:2.09	1:1.89
VI ³	24	33.9	8.0	1:1	1:1.78	1:1.14
XLV ³	28	52.1	8.0	(1:1.41) ⁵	(1:3.75) ⁵	(1:2.16) ⁵
LXXIX ¹¹	32	95.0	14.0	1:1.17	1:1.53	1:1
S ⁴	40	140.0	21.0	1:1.14	1:1.83	1:1.75
XXXIV ³	80	366.0	50.0	1:1.25	1:1.47	1:1.50
XLVIII ³	130	574.0	86.0	1:2	1:2.61	1:2.37
Infant ⁹	334	2100.0	555.0	1:1.63	1:1.57 ⁸	1:1.70
Adult	850	5372.0 ¹⁰	1500.0	1:2.57	1:3.66	1:6.45
				1:2.55	1:2.56	1:2.70

¹ As measured on the border opposite the mesenteric attachment.

² Vertex-breath measurement in fetuses. Vertex coccygeal measurement after birth.

³ After Mall. ⁴ Wisconsin collection. ⁵ Comparing Embryos IX and VI.

⁶ Comparing fetuses XLV and S; duodenum 3.6: 7.27, jejunum (coils 2 and 3) 8.8: 56.4, ileum (coils 4, 5 and 6) 39.7: 74.5.

⁷ Comparing fetuses S and XXXIV; duodenum 7.27: 11, jejunum (coils 2 and 3) 56.4: 89, ileum (coils 4, 5 and 6) 74.5: 266.

⁸ Comparing fetuses XXXIV and XLVIII; duodenum 11:22, jejunum (coils 2 and 3) 89: 132, ileum (coils 4, 5 and 6) 266: 420.

⁹ Average after Weinberg.

¹⁰ Average after Sernoff.

¹¹ After Mall, Anat. Anz., Bd. 16, 1899.

The relative rate of growth of body axis is somewhat less than that of the intestines in embryos between 17 and 24 mm. or less. There then begins a relatively very rapid development of the coils of the small intestines within the umbilical cord. In one fetus of 24 mm. these are nearly twice as long as in another (compare x and vi in the table). The relatively more rapid growth of the small intestines continues in fetuses between 24 and 28 mm. in length, while the rate of growth of the large intestines still continues to be about equal to that of the axis of the body. During the next stage, however, in fetuses between 28 and 40 mm. in length the small intestines grow nearly twice and the large intestine over twice as fast as the axis of the body. As may be seen from footnote 6 of the table, during this period the duodenum and the jejunum (abdominal part of the small intestines, primitive loops 1 to 3 of Mall) grow much more rapidly than the ileum (umbilical coils, loops 4 to 6 of Mall). The relatively great growth in length of the large intestines is due to the development of the left colic loop. After the return of the umbilical coils into the abdominal cavity, in fetuses between 40 and 80 mm. in length, both the large and small intestines grow somewhat more rapidly in length than the axis of the body, but the difference is far less marked than in the preceding period. The ileum (loops 4, 5 and 6 of Mall) grows relatively much faster than the jejunum (loops 2 and 3 of Mall) according to the data furnished by Mall. From this time on, although there are undoubtedly great individual differences in the growth in length of the intestines, on an average there does not appear to be much difference in the growth in length of the intestines as compared with that of the axis of the body, with the exception of a great increase in the length of the large intestine toward the latter part of fetal life.

From table 1 it is clear that the period of relatively greatest intestinal development is that immediately preceding and accompanying the return of the umbilical loop into the abdominal cavity, the period when the fetus is growing from 20 to 50 mm. in length. This is the critical period in the development of the intestines. Variations which occur later are of minor importance.

PLATES

PLATE 1

EXPLANATION OF FIGURES

1 and 2 Ventral and lateral views of a reconstruction of the stomach, small and large intestines, rectum, bursa omentalis and mesentery, of a human fetus 27 mm. long (No. 6, Wisconsin Collection) 7 diameters.

3 and 4 Ventral and lateral views of a reconstruction of the stomach, small and large intestines, rectum, bursa omentalis and mesentery of a fetus 35 mm. long (No. 8, Wisconsin Collection). Owing to an artefact the coils in the umbilical cord were so injured that an accurate reconstruction could not be made. In figure 3 this portion of the intestines is not shown. In figure 4 a schematic reconstruction of this portion of the intestines is given. 7 diameters.



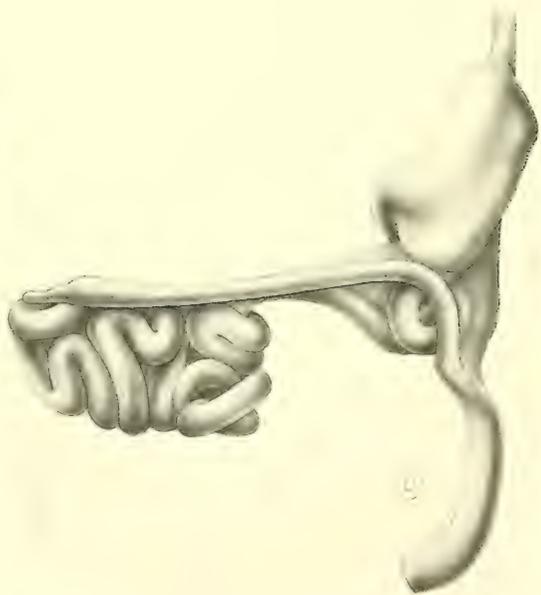
1



2



3



4

PLATE 2

EXPLANATION OF FIGURES

5 and 6 Ventral and lateral views of a reconstruction of the stomach, small and large intestines, bursa omentalis and mesentery of a fetus 40 mm. long (No. 8, Wisconsin Collection). In figure 6 the kidney and adrenal gland are also shown. 5 diameters.

7 Ventral view of a reconstruction of the stomach, duodenum, caecum, colon, rectum, bursa omentalis and mesentery of the same fetus. 5 diameters. The specimen here illustrated may be compared with that described by Mall (Supplementary note on the development of the human intestine; *Anat. Anz.*, Bd. 16, pp. 5, 492, 1899).

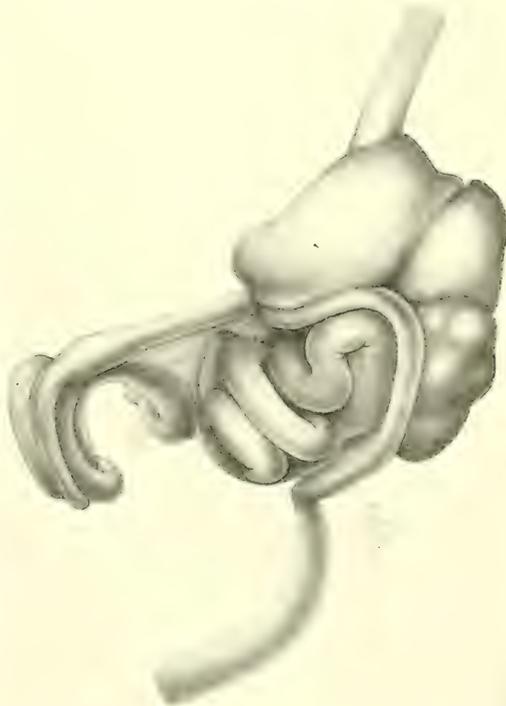


PLATE 3

EXPLANATION OF FIGURES

8 and 9 Views of the intestines in a subject in which there was a large splenic flexure and a transposition of the sigmoid colon to the right side of the body. In figure 9 the small intestines have been removed. The body in which the conditions here illustrated were found was that of a boy five and one-half years old who had died of chronic Bright's disease in an institution for the feeble-minded. No other marked abnormalities of development were noted. The position of the intestines is shown in the figures. The mesentery of the transverse mesocolon was short. The mesentery of the small intestines was attached to the body wall in essentially the normal position. The descending colon was in part fused to the left side of the mesentery of the small intestine. The great omentum extended downwards from the greater curvature of the stomach over the transverse colon and beneath the loop of the splenic flexure to the lower border of which it was attached.

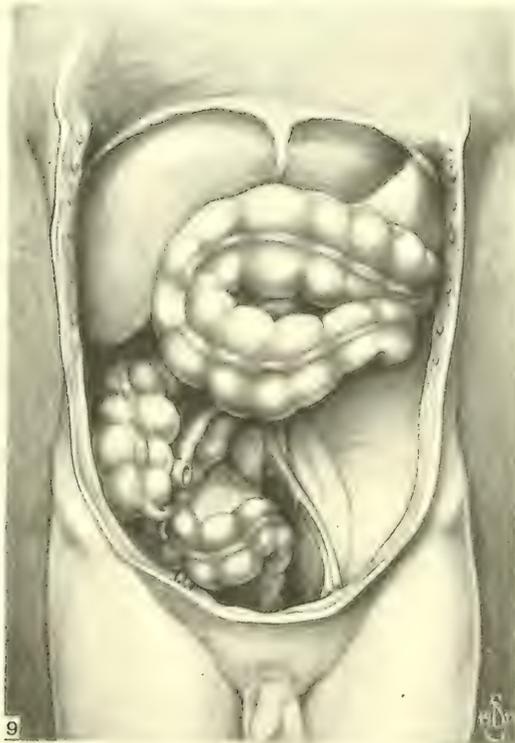
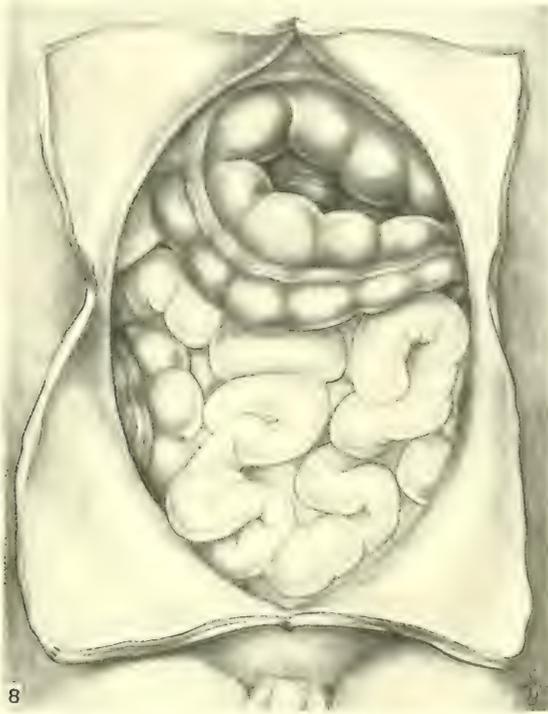
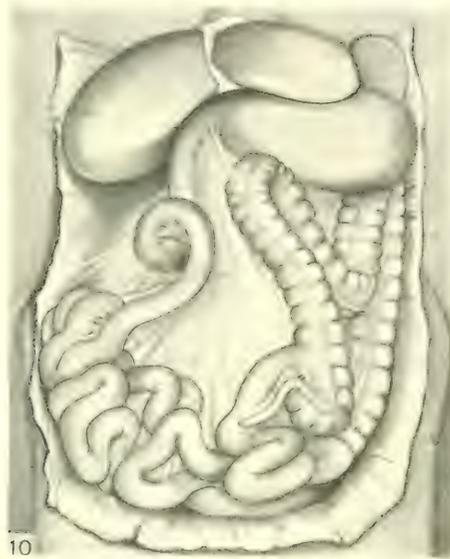


PLATE 4

EXPLANATION OF FIGURES

10 After Huntington, *Anatomy of the peritoneum and abdomen*, figure 121. To illustrate a case of good development of the left colic loop, arrested development of the duodeno-jejunal loop and non-rotation of the entero-colic loop.

11 After Strehl, *Archiv f. klin. Chirurgie*, Bd. 87, 1908, cited by Broman, *Normale and abnorme Entwicklung des Menschen*, figure 326. To illustrate a case of good development of the left colic loop, arrested development of the duodeno-jejunal loop and reverse rotation of the entero-colic loop.



THE EARLIEST BLOOD-VESSELS IN MAN

JOHN LEWIS BREMER

Harvard Medical School, Department of Anatomy

ELEVEN FIGURES

In a former publication¹ it was shown that in a rabbit embryo of five segments the intra-embryonic vascular arrangement consists of a net of solid endothelial cords, with occasional expanded portions, called angiocyts, in which a lumen is present. The net occupies the area, just dorsal to the entoderm, between the lateral border of the embryo proper, where it connects with the yolk-sac net, and the site of the future aorta, on either side of the neural groove. At the mesial border of the net numerous longitudinal anastomoses indicate the position of the future aorta: these anastomoses are not complete, so that the aorta is in three sections, not connected into a longer vessel. In the embryo then figured the shape of the meshes of the net, the fact that each section of the aortic net is separately connected with the lateral net, and the further fact that in the posterior part of the embryo the mesial border of the net lies progressively farther and farther from the median line—all these facts seem to me to point to the invasion of the embryo by this endothelial net, the actual growth of the endothelium of the yolk-sac vessels into this new territory. Both before the publication of this article and more recently, other investigators have cast some doubt on this conclusion by experiments on growing chick embryos, in which the heart, the aorta, and other vessels are found to develop on both sides of the embryo, even after the destruction of the yolk-sac vessels of one side. Since the present paper may help to throw some light on the points at issue, I shall defer a discussion till later.

¹ Bremer, J. L. *Am. Jour. Anat.* Vol. 13, p. 3, 1912.

It was with the extension theory in mind, therefore, as opposed to the ideas of many authors that endothelial cells arise in situ from mesenchymal cells in various parts of the body, that this investigation was undertaken to trace the origin of the vascular endothelium in man, and to locate the anlagen of the earliest blood-vessels. Heretofore it has been generally supposed that in man, as in other vertebrates, the first endothelial anlagen appear as the angioblast in the yolk-sac, between the entoderm and the splanchnic mesoderm. Opposing views have been expressed as to the part the two layers play in the formation of the blood-islands, which since the time of His have been recognized as, in part at least, the fore-runners of both blood corpuscles and endothelium; some authors maintain that the vascular cells are derived from the mesoderm, others that they are metamorphosed entodermal cells. The early vascularization of the chorion and body-stalk in man, before the presence of intra-embryonic vessels, and before the formation of somites, has long been noted, but usually considered as evidence of a very rapid growth from the yolk-sac anlagen.

In young human embryos, with the medullary plate of about 1 mm. in length, and with recognizable yolk-sac vessels, several authors have described, in the chorion, chorionic villi, and body-stalk, irregular spaces in the mesoderm, some lined with endothelium, some without definite lining; and recently Grosser² and Debeyre³ have separately mentioned, beside the irregular spaces, true blood-islands in the body-stalk, near the allantois.

In still younger embryos, with no vessels or blood-islands in the yolk-sac, Jung⁴ and later Herzog⁵ have called attention to accumulations of cells, sometimes arranged around a lumen, situated at the periphery of the mesoderm of the yolk-sac and body-stalk, bordering the extra-embryonic coelom. Jung's description of these is as follows, p. 104:

Auch finden sich (in the embryonic mesoderm) keine sicher zu erkennenden Gefässe. Allerdings sieht man an der in die Exocoelomhöhle hineinragenden Peripherie des Mesoblastes mehrfache Zell-

² Grosser, O. Anat. Hefte. Bd. 47, p. 649, 1913.

³ Debeyre, A. Journal de l'Anat. et de la Physiologie. Vol. 48, p. 448, 1912.

⁴ Jung. München. med. Wochenschr. Jahrg. 54, p. 1343, 1907.

⁵ Herzog, M. Am. Jour. Anat. Vol. 9, p. 361, 1909.

häufungen, welche sich stellenweise in Kreisform zu lagern, und ein Lumen in ihrer Mitte frei zu lassen scheinen. Auch findet man ähnliche Gebilde in den seitlichen Partien des Haftstieles, allein ich wage nicht zu entscheiden, ob es sich hier etwa um die ersten Gefässanlagen handelt. Jedenfalls sieht man nirgends in diesen kreisförmigen Gebilden einen Inhalt, geschweige denn etwa Dinge, die an Blutkörperchen erinnern könnten.

In his drawing (Fig. 17) these rings of cells are shown both on the yolk-sac and on the body-stalk. Herzog (p. 373) finds similar appearances "around the allantois stalk where its mesoderm is continuous with the yolk-sac mesoderm." "These formations undoubtedly represent the earliest anlagen of the yolk-sac blood-vessels." Herzog's interpretation of these cellular rings I have found to be incorrect, but his mention of them helped to point to the location of the blood-vessel anlagen.

The embryos especially studied in this investigation are: (1) one of about 1 mm. (no. 825 of the Harvard Embryological Collection, fixed in Zenker's fluid, 10 μ sections cut in paraffin, stained in borax carmine and orange G); (2) Grosser's embryo, of practically the same age as the preceding (fixed in picric-sublimate, 10 μ sections cut in paraffin, stained in paracarmine); and (3) Herzog's embryo (fixed in Zenker's fluid, 7 μ sections cut in paraffin, stained in hematoxylin and eosine).

Beside these three I have examined the embryos described by Debeyre, Frassi,⁶ Dandy,⁷ Bryce-Teacher,⁸ and many others.⁹

In the Minot embryo, by the graphic reconstruction method, it is easily seen that on the yolk-sac, between the entoderm and the mesoderm, there is a net of vascular tissue, one-layered and consisting of blood-islands, solid cords and angioecysts. This net is best developed, and the blood-islands in it are located exclusively, in the hemisphere of the yolk-sac opposite the embryonic shield; this fact was pointed out by Minot in his article on the development

⁶ Frassi, L. Arch. mikr. Anat. Bd. 70, p. 492, and Bd. 71, p. 667, 1907-08.

⁷ Dandy, W. E. Am. Jour. Anat. Vol. 10, p. 85, 1910.

⁸ Bryce, T. H. and Teacher, J. H. Memoir, 1908.

⁹ I wish to express here my most sincere thanks to those who have given me ready access to much of the material used either for the substance of this paper or for valuable comparisons—to Professors Bryce, Debeyre, Grosser, Keibel, Kollmann, Mall, Robert Meyer, and Teacher.

of the blood in the Keibel and Mall "Human Embryology," where it is spoken of as suggesting the area opaca and area pellucida of lower forms. But, although the vascular net is much more prominent near the distal pole of the yolk-sac, the net of slender solid cords can be traced, in the area pellucida, nearly to the embryonic shield. Before reaching the shield, the net in this embryo comes to an end, though apparently unconnected cords, resembling the angioblast cords, may be seen here and there running for only short distances, extending even into the body-stalk.

In the chorion and chorionic villi of the same embryo there are many of the "irregular spaces" described by other authors; they are cavities in the mesoderm, with distinct walls formed either by flattened or spindle-shaped cells, or merely by the clean cut edge of the loose mass of mesenchymal processes and fine fibrils in which they lie. In single sections the larger cavities may appear absolutely empty of any cellular content, but on reconstruction each cavity is always found to contain a shred of tissue, apparently floating in it. Not infrequently these shreds enclose small vacuoles, or may even open out into angiocysts, with a single layer of cells forming the wall. They occupy only a small part of the mesodermal cavities, as a rule, and seem to be loose in them, like a thread run through a pipe; often they lie close against the walls of the cavities. The impression received from the study of these shreds is that of an endothelium shrunken away from the rest of the wall of a vessel. By making graphic reconstructions of these inner shreds of tissue, I found that here too the pattern obtained is that of a net, differing however from that on the yolk-sac by extending in three planes, or being in several layers. The reconstructions are not complete, in that many of the smaller branches were not traced to their termination, since it was chiefly desired to emphasize the net character of these cords.

This characteristic arrangement of these cords and vesicles, their general resemblance to other early endothelium, and other facts to be brought out later convince me that we are here dealing with the angioblast, or, perhaps better expressed, the endothelium of future blood-vessels.

The net in the chorion can be traced into many of the chorionic villi, and into the body-stalk as shown in figure 1; but in the body-stalk there is a great difference in the character of the elements composing it. The shreds or cords lying in relatively large mesodermal clefts open out into large endothelial angiocysts, from which cords again lead directly into large spaces in the mesoderm, without separate endothelial lining, or else end abruptly. I shall speak later of the histological appearances of these spaces, and for the present call them the "unlined spaces." Other cords, not connected with the net, can be traced further toward the embryonic shield, along the allantois, but there is no direct connection with the yolk-sac net. The unlined spaces and the terminal cords are always near the surface of the body-stalk.

Grosser first called special attention to the epithelial layer of mesodermal cells, the mesothelium, which forms the coelomic surface of the yolk-sac and of the body-stalk in his 1 mm. embryo, ending abruptly at the junction of body-stalk and chorion. Moreover, he pointed out that this mesothelium, instead of forming a smooth surface, dipped in irregularly, giving in sections the appearance of festoons. This disposition of the mesothelium is found in all the young human embryos which I have studied, except the Bryce-Teacher ovum, in which there is as yet no coelomic cavity. A model of the body-stalk of the Minot embryo, in which the mesenchymal core is left out, leaving the mesothelium as a sheet of wax, enclosing the vascular net, shows the outer or coelomic surface formed of rounded ridges and irregular mounds, between which are deep clefts or depressions; on the inner surface of the mesothelium these clefts are seen as sharp ridges or pointed funnel-shaped ingrowths. The model shows clearly that these ridges and ingrowths touch here and there, and are apparently continuous with, either the angioblast cords or the unlined spaces of the vascular net.

Through the great generosity of Professor Grosser I was enabled to repeat my reconstructions from his embryo, which is faultlessly preserved, stained, and sectioned. The results correspond with the findings in the Minot embryo, except that in Grosser's embryo it is possible to trace the net from the chorion through the body-

stalk further toward the yolk-sac, and to follow one cord in its tortuous course to its junction with the yolk-sac net. Other cords from each net are apparently reaching out toward the other, some having nearly spanned the intervening space (figs. 2 and 3). There are no cords unconnected with either net, such as are found in the Minot embryo. The end of the chorionic net nearest to the yolk-sac is composed of cords and unlined spaces, with no endothelial angiocysts.

A careful study of the unlined spaces in this embryo leads to more convincing proof that they and the mesothelial ingrowths are actually connected. In the Minot embryo such connections can be made out only by reconstructions, whereas in the Grosser embryo the plane of section has fortunately, in several instances, shown the whole connection in a single section, as shown in figures 6 and 7. Cells resembling young blood corpuscles may be very infrequently found in the unlined spaces, and even in the mesothelial ingrowths.

The blood-island described by Grosser as lying in the mesenchyma of the body-stalk of his embryo, is found to be connected by a protoplasmic cord, resembling an angioblast cord, with the vascular net (fig. 2). In the Minot embryo a few smaller but otherwise similar groups of cells are found, one of which is shown (fig. 1) connected directly with an inpocketing of the mesothelium. In both embryos the cells of the islands are rather small, rounded, with little protoplasm, resembling in these respects the cells of the young islands of the yolk-sac. In the body-stalk of the Dandy embryo though it is much older than the two under consideration, I have found similar islands, one directly connected with an inpocketing of the mesothelium but not with the blood-vessels, the other smaller one, of about 20μ in diameter, with no present connection with either blood-vessel or mesothelium.

In the embryo described by Professor Debeyre which he also most kindly allowed me to examine, the relations of mesothelium, unlined spaces, cords, angiocysts, and blood-island appear to be the same; but no reconstructions were made.

Herzog, it will be remembered, described in his embryo certain rings and small groups of cells, lying in the coelom at the edge of

the body-stalk, which he considered the anlagen of the yolk-sac vessels. This valuable very young embryo has been kindly given to the Harvard Embryological Collection, so that I have had the opportunity to study it carefully; and in spite of fact that the unfortunate breakage of many sections makes complete reconstructions impossible, enough can be seen to give a sure basis to the following account of its vascular anlagen. After the study of the Minot and Grosser embryos it is not difficult to recognize that the groups and rings of cells in question are in fact tangential sections of the mounds of the mesothelial sheet covering the body-stalk; the lumen of the rings is the very loose mesenchymal core of the stalk. As is seen in the drawing (fig. 4) the mesothelial covering is not complete in this embryo, but leaves large areas of the surface uncovered, where the processes of the mesenchymal cells of the core, with numerous intervening fibrils, form the only border between the body-stalk and the coelom, as is the case along the inner border of the chorion proper. Where the mesothelial cells are present they occasionally project into the core of the body-stalk, lining a funnel-shaped diverticulum of the coelom, as is seen also in the older embryos. Mitotic figures are frequent at such points. The inner end of such projections are continued, usually in a curved direction, as irregular hollow spaces (fig. 4, *a*), as cords or as small groups of cells, without lumen. One of these cords, three or four cells long, runs in the chorion parallel to its inner border. It seems very probable that from the cell groups other cords also run in the chorion, but the destruction of certain sections makes positive proof of this impossible. One especially large and well defined group of cells lies at the base of the body-stalk, in the mesenchyma near the coelomic border, and directly over it is found a mesothelial sheet and inpocketing; but here again the condition of the sections makes it impossible to affirm that the two structures are actually continuous. In the chorion there are none of the large irregular spaces seen in older specimens; but, extending from the base of the body-stalk for a considerable distance, perhaps a quarter of the way around the chorion, are cords of cells which, on reconstruction, are found to form an irregular net, in one plane, parallel to the surface of the coelom. This net shows no

trace of angiocysts, nor are there even vacuoles in the cell protoplasm. The cells forming it are broader, or thicker, than the surrounding mesenchymal cells, many of which are spindle-shaped, and the cord cells usually lie in well defined, though small, cavities among the mesodermal fibrils and cell processes (fig. 5).

The yolk-sac of the Herzog embryo is covered by a mesothelium, apparently originally complete, though broken now in many places but this mesothelium lies everywhere close against the enclosed entoderm, and shows no signs of the funnel-shaped ingrowths such as are found around the body-stalk. In Jung's paper, however, (his fig. 17) the mesothelium of the yolk-sac is shown as quite far separated from the entoderm, and in at least two places in the one drawing (the only section of the embryo proper figured by him) the mesothelium of the distal pole is arranged in what appear to be typical funnels.

In the Bryce-Teacher ovum, which was also most generously placed at my disposal for examination, the mesodermal cells are often spindle-shaped, and may be arranged in chains of two or three cells, followable from one section to another. But on reconstruction these cells do not form a net, except by the very finest protoplasmic processes, as in ordinary mesenchyma. A coelom is absent, and therefore there is no mesothelial surface. Though the chains of cells look somewhat like angioblast cords, I am satisfied that they are only portions of the general mesenchyma, especially since similar chains of spindle-shaped cells, followable for only short distances, and not connected with the vascular net, are to be found in the mesenchyma of older embryos, even at the time when the blood-vessels are well established. Much larger, more distinct cords of cells in the mesoderm of this ovum are found to be processes of the surface ectoderm, of which a few cords traverse the mesoderm of the future chorion. Similar, but hollow, "chorionic canals" are described by Grosser and by F. T. Lewis¹⁰ in older embryos. From my study of this ovum I should say that no vascular anlagen exist at this stage.

¹⁰ Lewis, F. T. Communication to the 30th Session of the Am. Ass. Anat., 1913.

I have mentioned the "unlined spaces" which seem to form a part of the vascular net, and wish now to describe them and the other portions of the net in some histological detail. In the Herzog embryo (fig. 4) a long funnel-shaped diverticulum of the coelom, bounded by a definite mesothelial layer, is seen to expand at its distal end (*a*). The two walls approach each other in the middle portion of the diverticulum, and are either in contact or definitely fused, thus cutting off a distal cavity. If the fusion of the walls had continued further toward the main coelom, the distal cavity would appear as an irregular space, deep in the mesenchymal core of the body-stalk, but connected with the mesothelial surface layer by a cord of mesothelial cells. Moreover, the cavity would be a portion of the coelom, and its bounding walls would be also mesothelium. If we turn now to the Grosser embryo, we see another cavity (fig. 6, *a*); the walls are histologically similar to the mesothelium (*mes.*) covering the body-stalk at this point, and are connected with the surface layer (at the top of the drawing) by a cord of the same type of cells. The funnel-shaped mouth of the original diverticulum is still clearly seen. Both on the coelomic surface and bounding the cavity, the protoplasm of this layer forms a narrow but definite sheet, and is connected by numerous processes with the underlying mesenchyma. The nuclei are broader than the sheet of protoplasm, and project now toward the cavity, now toward the mesenchyma. The mesothelium of the surface, the mesothelial cord, the walls of the enclosed cavity, and the surrounding mesenchyma all form a syncytium, as no cell walls are present. The shape of this cavity as traced through the sections and the fact that in other sections there are other mesothelial cords connecting it with the surface make it probable that several smaller cavities have coalesced to form this one. A much smaller cavity is seen in the same drawing at *b*, near the surface.

Another similar cavity, also from the Grosser embryo (fig. 7), shows the same characteristics as the last described, except that the funnel-shaped opening into the coelom has apparently become obliterated by a more complete fusion of its walls, so that the mesothelial cord now springs from the under side of a smooth

portion of the surface layer. Here again the walls of the cavity are histologically similar to the boundary of the coelom and to the mesothelial cord. In this case the walls are continued in two directions by protoplasmic strands (*c* and *d*) broader than the ordinary mesenchymal processes, with which they connect, and of the same character as the strand joining the walls of the cavity with surface mesothelium. One of these (*c*) leads to a second funnel on the surface, and merely proves the coalescence of two separate ingrowths; the other (*d*) ends blindly, as is shown in the drawing, and indicates either that the distal, as well as the proximal end of the original coelomic diverticulum may be obliterated by fusion, or (which seems to me more probable) that the walls of the diverticulum have the power of further growth. In figure 6 another similar strand is seen at *c*, which in this section appears isolated, but by reconstruction is found to connect two adjacent cavities.

The relation between these unlined spaces, which from my drawings I can only consider as isolated portions of the coelom, and the angiocysts which have a definite endothelial lining and an extra-intimal space, perhaps due to shrinkage, is indicated in the next two drawings. One, from the body-stalk of Grosser's embryo, (fig. 8) shows the typical mesothelial wall on one side of the cavity, and on the other an apparent delamination of an inner layer, continuous at either end with the mesothelial wall, but separated as a whole by an extra-intimal space from the underlying layer, which is still an integral part of the surrounding mesenchyma. The other drawing (fig. 9) is from the Minot embryo, near the wall of the coelom at the side of the body-stalk. Two funnel-shaped diverticula from the coelom lead toward the inner cavity, though the mesothelial cords are not so distinct as in the former cases. The wall of the cavity is mesothelial at the lower left-hand corner, but is continued as an inner lining, with an extra-intimal space. This inner lining, in the form of an extremely thin sheet of tissue containing scattered nuclei, running in part obliquely through the section, in part directly away from the eye of the observer, sends out, in three directions, processes which connect with the inner lining of other similar cavities; one such

connection is shown in the drawing, the others are continued in adjoining sections. We are dealing here, undoubtedly, with endothelium, and the processes are typical angioblast cords. The cords run usually, if not always, in well defined spaces in the mesenchyma.

The mesothelial cords, as well as the processes of the surrounding mesenchymal cells, are left, as it were, attached to the outer wall of the cavity, while the endothelium lies free within. Such mesothelial cords are found in much older embryos, as for instance in that described by Dandy (of seven segments) and in one of about the same age kindly lent me by Prof. R. Meyer of Berlin. They lead from the outer walls of the now well defined umbilical vessels to funnel-shaped irregularities of the coelom wall, where they are continuous with the surface mesothelium, frequently anastomosing with each other. Since they seem involved in the process of haemopoiesis, I shall mention them again later.

In addition to the method just described, whereby endothelium is derived by delamination from the mesothelium of isolated portions of the coelom, a more direct method seems to be shown in the preparations studied. It will be remembered that in the reconstructions of the Minot embryo (and the same is true of those of the Grosser embryo) the angioblast cords of the net could be frequently traced to ingrowths from the coelomic mesothelium without the intervention of the unlined spaces. Isolated portions of such cords, in advance of the net in the Minot embryo (fig. 1) are also thus connected. Again, in the Herzog embryo, one of the funnel-shaped ingrowths is continuous, as seen by reconstruction, with a short cord (fig. 4, *b*) which it is impossible to follow far, but which is similar to those forming the net in the chorion. These cords are characterized by rather darkly staining protoplasm, and by the absence of protoplasmic processes connecting them with the surrounding mesenchyma, from which they are usually slightly separated, leaving an extra-intimal space (fig. 5). Their resemblance to the endothelial cords which form the links between angiocysts in all the vascular nets studied makes me believe that they also are true endothelium, and that thus endothelium may

arise directly as an extension of the mesothelial cords, without the process of delamination. Any future cavity in these cords would be potentially a part of the coelom.

If these interpretations of the sections are correct, true endothelium may arise in two ways from the mesothelium. Certain appearances make it at least probable that blood corpuscles may also be a product of the same tissue. In the yolk-sac blood-islands it has long been agreed that both endothelium and corpuscles come from the same anlagen. In the body-stalk net of the Minot embryo, which is not connected with the yolk-sac net, there are a very few blood-corpuscles, or at least free cells within endothelial cavities. In the Grosser embryo, in which the connection of the two nets is apparently solid, there are also a very few corpuscles. In both of these embryos the yolk-sac corpuscles are limited to the distal pole; it seems certain, therefore, that the few blood-corpuscles in the body-stalk vessels must have developed in the net itself. The blood-islands already mentioned in the body-stalks of these two embryos are either not connected with the other vessels, or are so connected only by apparently solid strands (figs. 1 and 2); they will probably later supply their quota of corpuscles, but do not seem to account for the few already present.

In the embryo of Professor Meyer, already described as showing mesothelial cords still connecting the blood-vessels with the coelomic wall, the blood-corpuscles are differentially stained, and easily distinguishable from other cells. They are found not only in the blood-vessels, but also in the mesothelial cords, of which they seem to form a part. Similarly in the drawing of the Grosser embryo (fig. 7) there are, in and connected with the mesothelium, cells whose nuclei resemble those of the three cells floating free in the cavity. Though these cells are not of the type of corpuscle which one would expect at such an early age, cells of the same character are found occasionally in the yolk-sac vessels of both the Grosser and the Minot embryos, and from their position I consider it probable that they are blood-corpuscles. We should, then, credit this mesothelium with the additional power of forming occasional corpuscles without the mediation of blood-islands.

Briefly summarized, my observations point to ingrowths of the mesothelial layer covering the yolk-sac and body-stalk as the anlagen of the blood-vessel endothelium and, to a lesser extent, of the blood-corpuses. The anlagen, though limited to certain areas commensurate with the extent of the mesothelial layer, are multiple and form a net by the growth and coalescence of the separate units. A further extension, accompanied by the incorporation of other units, effects the union of the body-stalk net with that on the yolk-sac. On the other hand, the net in the chorion and chorionic villi seems to me to be formed by direct extension from the body-stalk, without the addition of new components, and to be the result of a centrifugal growth of endothelial sprouts, in the form of angioblast cords, with here and there expanded angiocysts, which advance through the mesenchyma. This would correspond with the fact that no surely isolated endothelial cords have been found in the chorion, that in the Herzog embryo the net extends only part way around the chorion and is centered at the base of the body-stalk, and that there is in the embryos studied no mesothelium on the inner surface of the chorion proper, and therefore no possibility of new anlagen there. The chorionic mesothelium is developed later, but only after the vessels are well established.

That the angioblast cords and angiocysts are direct sprouts from the endothelium and not mesenchymal cells metamorphosed in situ and added to the growing tip is shown (aside from the recent work of Clark¹¹ and others on endothelial growth) by the presence of the clear-cut extra-intimal space (figs. 10 and 11) and by the fact that there are no protoplasmic connections between endothelium and mesenchyma, even in the most distal vessels. The irregularities occasionally found in the outlines of the endothelium seem to me to point to its contraction or perhaps to amoeboid movements incident to the formation of new sprouts. That the extra-intimal space may be an artefact, that the so-called cords may be in reality collapsed tubes, is, to my mind, immaterial, and awaits proof by injection methods; the sharp boundary between

¹¹ Clark, E. R. *Am. Journ. Anat.* Vol. 13, p. 351, 1912.

the endothelium and the mesenchyma through which it runs points to a difference in origin between the two tissues.

We have not yet attained a sure histological basis for differentiating endothelium in ordinary specimens, nor is it perhaps to be expected in tissues as young as those under discussion. In a recent paper Clark¹² described certain differential characteristics of endothelial nuclei, after special fixation and stains, but this is in chicks of a relatively older stage than the present human material, and I could find no trace of such differences in younger material and with the common stains used. In the Grosser embryo, as shown in many of the drawings, endothelium and mesothelium are both often marked by the presence of very fine intra-cellular fibrils, absent in the mesenchymal cells; these fibrils are not found in the Minot embryo, nor in any of the others studied, owing probably to differences in the fixing fluid employed. They cannot, therefore, be used as a general distinguishing sign.

The coincidence of the views forced upon me by my observations with the now ancient theory of His,¹³ Bütschli,¹⁴ and others, that blood-vessels are in some way related to the coelom, is apparent, and the significance of this when correlated with the facts known of the inter-relation of the blood-vascular system and the coelom in certain invertebrates, must strike anyone who is interested in phylogeny.

This has been briefly noted by Huntington¹⁵ who found, in a cat embryo of 10 mm., a "clearly limited and well defined funnel-shaped stoma, occupying the dorsal extremity of the coelomic cleft and apparently opening directly into the spaces of the early lymphatic plexus" (p. 26), without, however, attaching more than a "suggestive" importance to the fact. It is also interesting to note that the origin of endothelium from the mesothelium adds to the already great number of structures derived by ingrowths from this source, among which may be mentioned the tubules of the pro-

¹² Clark, E. R. *Anat. Record*. Vol. 8, p. 81, 1914.

¹³ His, W. *Abhandl. math. phys. Classe K. Sächs. Ges. Wiss.* Vol. 26, p. 173, 1900.

¹⁴ Bütschli, O. *Morph. Jahrb.* Bd. 8, p. 474, 1883.

¹⁵ Huntington, G. S. *Memoirs Wistar Institute*, No. 1, 1911.

nephros (indirectly also those of the mesonephros), the sex cords, and the cortex of the suprarenal gland, according to the later views.¹⁶

The origin of the chorionic vessels from the mesothelium of the body-stalk explains the fact, mentioned by Knoop¹⁷ in anomalies of the amnion, and by Bauereisen¹⁸ in haematomoles, that vessels can exist in the chorion "without the help of the umbilical vessels."

Let us now turn to a discussion of the papers before referred to, in which the authors describe the results of experiments, on chick or other bird embryos, undertaken to prove the origin in situ of intra-embryonic blood-vessels, as opposed to the extension or growth of such vessels from some extra-embryonic source, by destroying or cutting off the whole or part of the area opaca of one side. Vessels on the injured side are obtained in many cases by all these investigators, after a further incubation of the operated embryos; and these vessels are generally in the position of aorta, heart, vitelline vein, and even other vessels of normal embryos. Where only part of the vascular system exists on the injured side, it appears to be always that nearest the mid-line; i.e., the aorta may be present without the heart, or the aorta and heart without the other vessels. Not infrequently the vessels are abnormally large, or the lateral vessels may be only roughly in the normal position. Gräper¹⁹ and Hahn,²⁰ though the results of each were known to the other before publication, come to different conclusions as to the origin of the vessels; the first maintaining from his experiments, an entodermal, the other from his experiments a mesodermal derivation. Miller²¹ is chiefly interested in the negative proof that the intraembryonic vessels do not reach their ultimate destination by direct ingrowths of endothelium from the lateral area, as was maintained in my former paper.

¹⁶ Goormaghtigh, N. Bull. Soc. Med. de Gand. Vol. 5, p. 24, 1914.

¹⁷ Knoop, H. Beitr. Geburtsh. Gynäk. Bd. 7, p. 284, 1903.

¹⁸ Bauereisen, quoted from Jagerroos, B. H. Archiv f. mikr. Anat. Bd. 82, Abt. 1, p. 271, 1913.

¹⁹ Gräper, L. Arch. f. Entw. Mech. Bd. 24, p. 375, 1907.

²⁰ Hahn, H. Arch. f. Entw. Mech. Bd. 27, p. 337, 1909.

²¹ Miller, A. M. Anat. Record. Vol. 8, p. 91, 1914.

From the differences in the conclusions reached by two of these authors it seems certain that more work should be done along these lines before a consensus of opinion can be expected. I wish to point out a few possibilities which should, I think, be considered in any such future work.

As was shown in the figures of my reconstructions of rabbit embryos, the vascular net has an irregular mesial border, certain strands lying further toward the midline than the position of the future aorta. Though in the younger embryos the extension of these strands across the median line of the embryo proper to form a net on the opposite side is rendered impossible by the close approximation of the medullary groove, notochord, and entoderm, yet long before the stage figured in many instances cited by these authors the mesoderm has grown across the median line, and might afford a pathway for endothelial sprouts from side to side. Another and earlier pathway is at the posterior end of the embryo, behind the primitive streak, where the mesoderm very early extends across the median line. The angioblast cords, by which connections from recognizable blood-vessels to apparently isolated angiocysts can be traced (if we accept, for the moment, and for the purpose of argument, the extension theory) are delicate strands, easily overlooked, and moreover may last only a few hours, if the mechanical conditions are not favorable to their continued development into vessels (cf. figs. 1 and 3, loc. cit.). It is not to be expected, therefore, that anything short of a very complete series of such operated embryos, fixed at progressively longer intervals of incubation after operation, can settle whether or not there is any extension from the opposite side.

I think it well at this point to define more accurately what I mean by angioblast cords, especially since I believe that their recognition may perhaps help to explain the frequently described endothelial spaces unconnected with any injectable vessels. The angioblast cords are apparently solid cords of cells, connected end to end or in small groups, running between the processes of the surrounding mesenchymal cells, when these are present, often touching them, without however actually fusing with them. The diameter of the cords is never as small as that of the mesenchymal

processes, though it is often less than that of the cord nuclei. The cords tend to form nets by anastomosis of larger mesh than the mesenchymal net, and angiocysts by vacuolization wherever space is given. They are usually sharply defined from the surrounding tissue, and may show an extra-intimal space. They must necessarily be extremely hard to recognize in dense mesenchyma, though easy to trace in perfectly prepared series of looser tissue.

A second possibility to be considered in attempting to explain, on the basis of the specificity of endothelium, the presence of vessels on the injured side of the operated embryos is advanced in this present paper. If the earliest human vessels arise from the mesothelium lining a portion of the extra-embryonic coelom, by multiple anlagen which later fuse; and if the intra-embryonic coelom, developed later, is also lined by mesothelium, the opportunity is perhaps offered for similar, but later, anlagen for intra-embryonic vessels. The instance cited by Huntington of a coelomic opening into the spaces of the early lymphatic plexus should be borne in mind. I am well aware that the yolk-sac blood-islands are not proven to arise from mesothelial anlagen, that in avian embryos they are even said to be present before the formation of the coelom in the area vasculosa, a fact that would point to some tissue other than mesothelium (perhaps a premesothelial stage of mesoderm) as that from which endothelium is derived; yet certain connections between the vessels of the operated embryos above referred to and the mesothelial wall of the coelom, shown in many of the drawings and seen by me in one of Miller's preparations, seem to me to make this possibility at least worthy of consideration in future study. It is easily conceivable that such separate anlagen might arise under abnormal conditions in positions where they are normally absent; or on the other hand it may be that multiple anlagen of intra-embryonic vessels in close relation to the coelom are normal, and that the net figured by me in this position is the result of their confluence. Yet the finding of no separate angioblast cords in advance of the general net in this specimen, especially at the younger caudal end, would militate against this latter proposition. It is also possible that embryos

of such different types as chick and rabbit or man may show differences in details of the vascular development.

Once given an endothelial net in the general area of the future vessels, mechanical forces would, as pointed out in the paper so often referred to, locate the portions of that net which are to remain and form aorta, heart, etc., and the portions which are to disappear because unfavorably placed. It is probable that all the variations in size and shape of heart and aorta, and even in position of the more lateral vessels, which are not infrequently shown in the drawings of the operated embryos, are due to changes in the normal tension of the germ layers, consequent on the injury. That these changes must be great is shown in gross by the bending of the whole body of such embryos away from the injured side. A study of these embryos from this point of view would lead to interesting results in the field of developmental mechanics.

CONCLUSIONS

In human embryos the earliest blood-vessels arise separately in the yolk-sac and in the body-stalk, by multiple anlagen.

The anlagen in the body-stalk (and perhaps also in the yolk-sac (cf. Jung's figure 17) are funnel-shaped ingrowths of the surface mesothelium, which is present as a definite layer only on the two areas mentioned. By partial fusion of the walls of an ingrowth a portion of the coelom, still bordered by mesothelium, may be cut off as a separate cavity, lying deep within the substance of the body-stalk.

The endothelium seems to arise either (a) by delamination from the walls of such a detached portion of the coelom, or (b) by direct extension, in the form of an angioblast cord, from the mesothelial ingrowth. From the endothelium, by whichever method developed, further extension is by means of the angioblast cords, which grow apparently through the surrounding mesoderm.

True blood-islands may occasionally arise by the multiplication of the cells of the mesothelial ingrowths, or scattered blood corpuscles may arise singly within these ingrowths.

Extension within the limit of the areas covered by the mesothelium is achieved by confluence of the detached portions of the coelom, or union of the cords; the result is a net comprising the various vascular units. Extension into the chorion, where the mesothelial layer is absent in the early stages, appears to be by direct centrifugal growth of the angioblast cords, without the addition of new elements from the surrounding mesenchyma.

The possibility that similar, but later, ingrowths from the mesothelium of the intra-embryonic coelom may give rise to intra-embryonic vessels, should be borne in mind in the study of such vessels, whether haemal or lymphatic.

PLATE 1

EXPLANATION OF FIGURES

1 Minot embryo, H.E.C. no. 825. Reconstruction of vessels in the body-stalk and chorion; connections with the mesothelium. *All.*, allantois; *bl.i.*, blood-island; *Coe.*, coelom; *Ect.*, ectoderm of chorion; *f.*, funnel-shaped connections, with unconnected cords; *Mes.*, mesothelium; *Vil.*, villus; *Ys.*, yolk-sac cavity. $\times 175$.

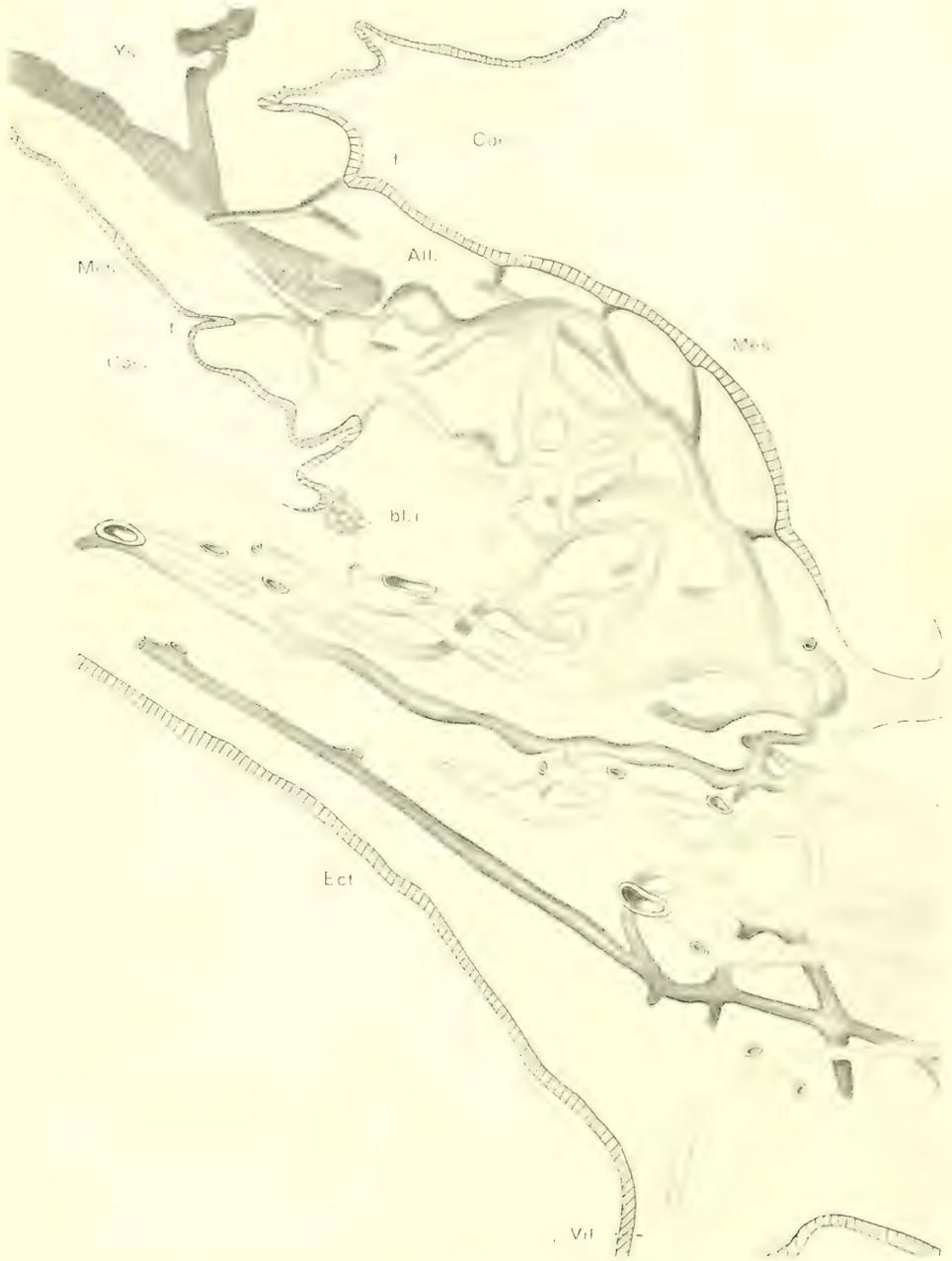


PLATE 2

EXPLANATION OF FIGURES

2 Grosser embryo, slide 3, row 5. Reconstruction of vessels in the body-stalk and chorion. Lettering same as in figure 1. $\times 275$.

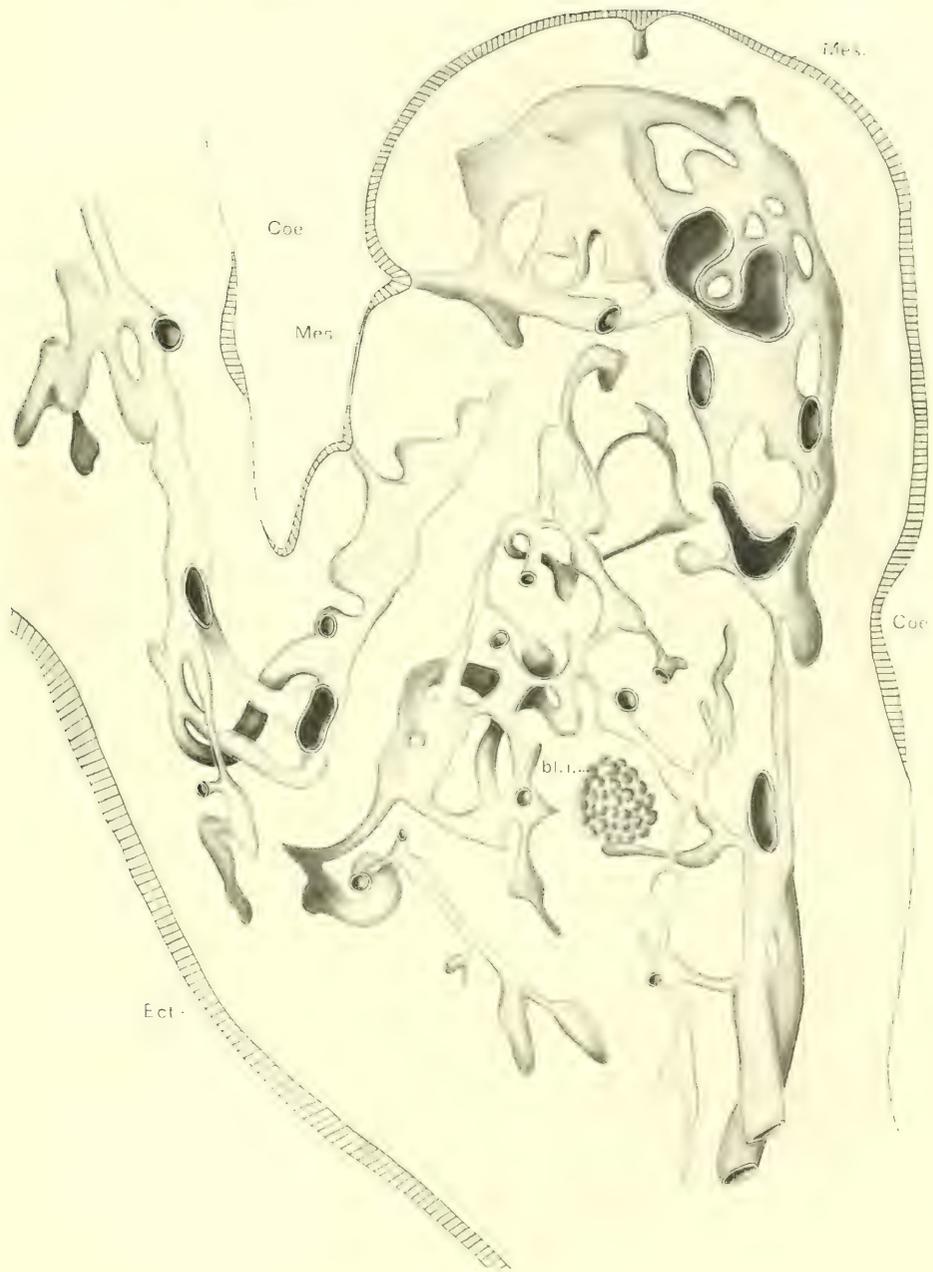


PLATE 3

EXPLANATION OF FIGURES

3 Grosser embryo, slide 3, row 3. Junction of body-stalk and yolk-sac, and connection of the two vascular nets, along the side of the allantois, between the mesoderm and entoderm. *All.*, cavity of allantois, looking into yolk-sac; *Am.*, cavity of amnion, which at one point is fused with the allantois; *Ent.*, sheet of entoderm forming wall of yolk-sac and continued as allantois wall; *Mes.*, sheet of mesothelium continued from yolk-sac to body-stalk; *Ys.*, cavity of yolk-sac. $\times 400$.

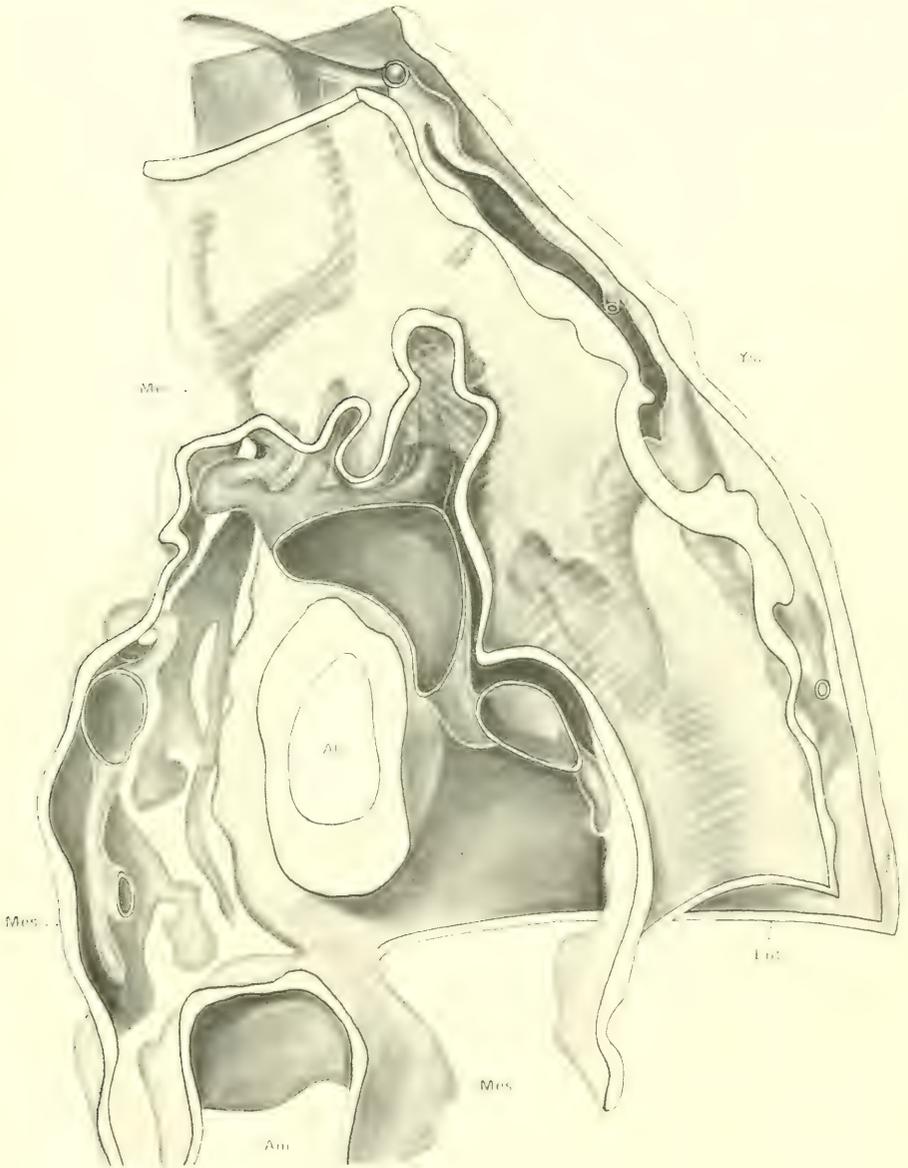


PLATE 4

EXPLANATION OF FIGURES

4 Herzog embryo, slide M, sect. 147. Chorion and base of body-stalk, to show irregularity of the coelomic surface, the partial mesothelial layer, and the funnel-shaped ingrowths (*f.*). The chorionic ectoderm has shrunken away, leaving naked the chorionic mesoderm, at the bottom of the drawing, and the stumps of two villi (*vil.*). *Coe.*, coelom; *a*, unlined space; *b*, angioblast cord. \times circa 800.

5 Herzog embryo, slide M, sect. 143. Angioblast cord in chorion, part of a net. Notice the clear-cut extra-intimal space. \times circa 800.

8 Grosser embryo, slide 3, row 8, sect. 3. Vessel from the body-stalk, to show delamination of endothelium and formation of extra-intimal space. \times circa 800.

11 Minot embryo, H.E.C. no. 825, sect. 27. Cross section of a strand of the net in the chorion to show a small angiocyst. \times circa 540.

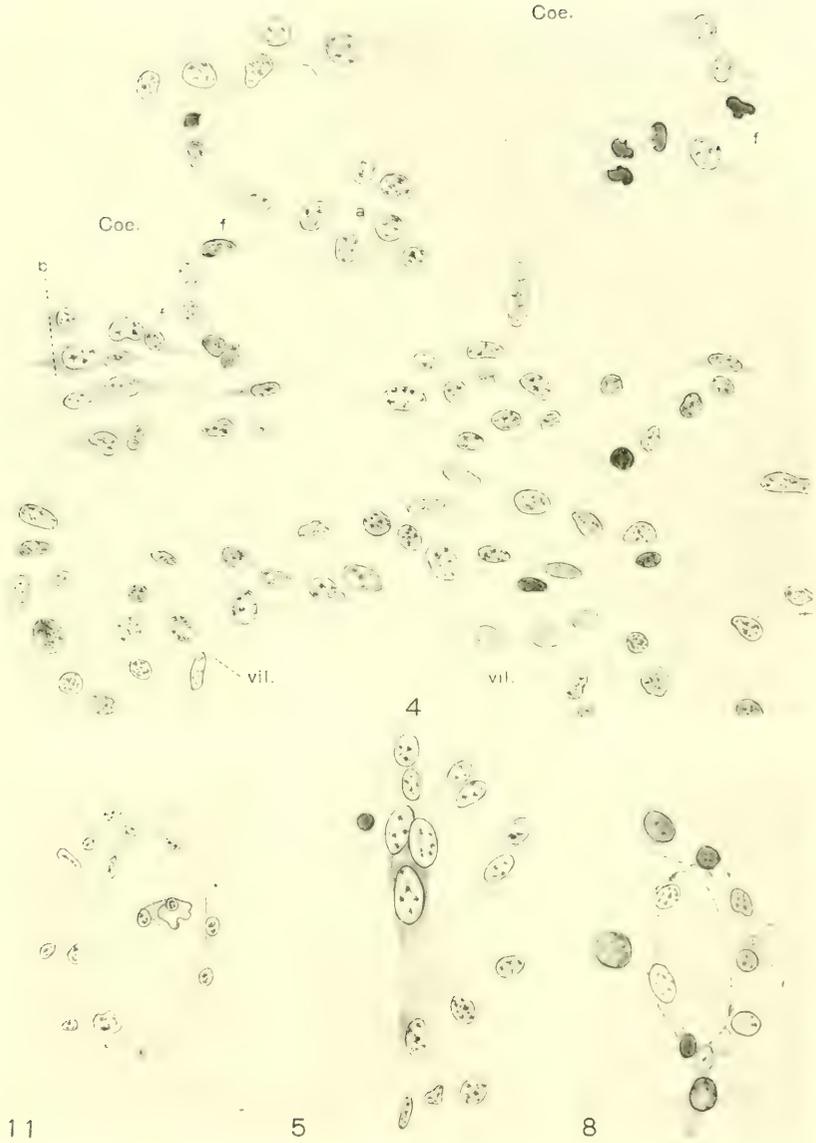


PLATE 5

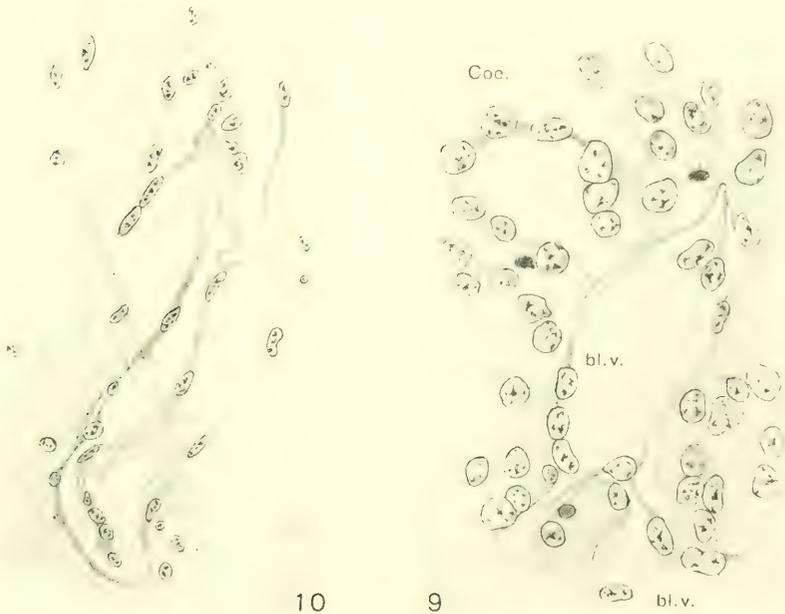
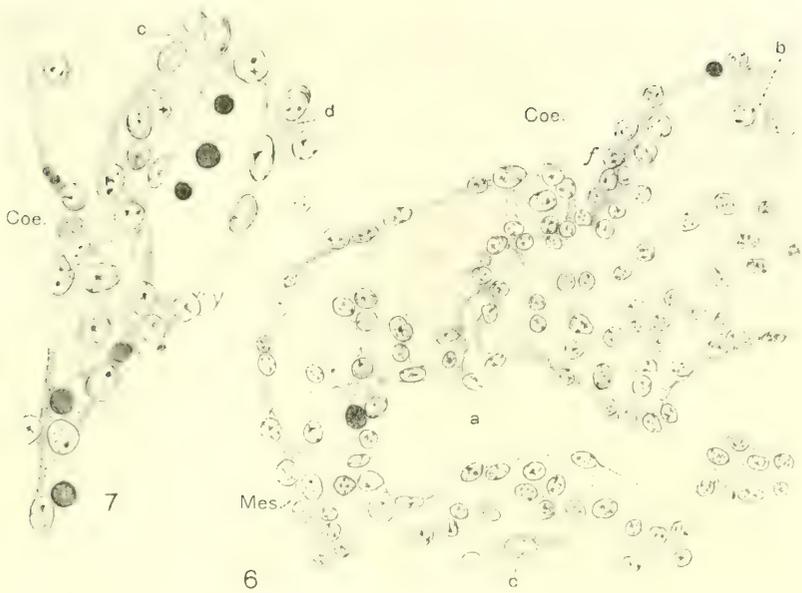
EXPLANATION OF FIGURES

6 Grosser embryo, slide 3, row 5, sect. 3. From the edge of the body-stalk, to show surface mesothelium (*Mes.*) and mesothelial cord, leading from funnel (*f.*) to unlined space (*a*). *Coe.*, coelom; *b*, smaller unlined space; *c*, endothelial cord. \times circa 580.

7 Grosser embryo, slide 3, row 4, sect. 5. From the edge of the body-stalk, to show mesothelial cord leading to unlined space, containing corpuscles. *c* and *d*, mesothelial cords (see text); *Coe.*, coelom. \times circa 800.

9 Minot embryo, H.E.C. no. 825, sect. 25. Vessel from the body-stalk, to show delamination of endothelium, and its extension as cords. Further description in text. *Coe.*, coelom; *bl.v.*, blood-vessel. \times circa 800.

10 Minot embryo, H.E.C. no. 825, sect. 24. Angioblast net in the chorion, to show the irregular contour of the cord, suggesting amoeboid movements, and the extra-intimal space even around the young branch. \times circa 540.



RETROGRESSIVE CHANGES IN THE FETAL VESSELS AND THE SUSPENSORY LIGAMENT OF THE LIVER

ARTHUR WILLIAM MEYER

From the Division of Anatomy of the Medical School of Stanford University

TWENTY-SIX FIGURES

The formation of the ligamentum teres and the persistence of the suspensory ligament of the liver in some mammals and not in others are closely related to some of the questions discussed elsewhere (Meyer '14). In text books, manuals and some monographs on the anatomy of the domestic and other animals it is usually stated that the thickened caudal border of the suspensory ligament of the liver represents the obliterated umbilical vein or round hepatic ligament. Krause ('84) writing on the anatomy of the rabbit adds, that it may occasionally remain patent. Reighard and Jennings ('01) state that in the cat "The ligamentum teres is the thickened free caudal border of the suspensory ligament. It is the remains of the fetal umbilical vein." Ellenberger and Baum ('91) writing on the dog, state however, that the suspensory ligament merely contains remnants of the umbilical vein (Ligamentum teres) and Martin ('02-'04) in his *Lehrbuch der Anatomie der Haustiere* says "In dem rechten Einschnitte (of the liver) ist die Gallenblase mit ihrem Gange eingebettet, in dem linkem zieht sich das Ligamentum teres hinein bis zur Nabelvenengrube." Chaveau ('90) also states that "At its (the suspensory ligament's) free border is a fibrous cord (the round ligament) formed by the obliteration of the fetal umbilical vein," and Milne-Edwards ('60) in the "Lecons sur la physiologie et l'anatomie comparee" declared that "On designe sous le nom de *ligament rond* le cordon fibreux qui resulte de l'atrophie de la veine ombilicale et qui loge dans l'epaisseur de ce repli pres de son bord libre."

These quotations it seems also represent the consensus of present opinion in this matter and yet anyone who has carefully observed the anatomy of the round and suspensory ligaments of the liver in the domestic animals must have been impressed with the fact that both are often entirely absent in comparatively young animals and at least partially even if not entirely so, in most very old animals. Moreover, it is more than probable that the statements quoted are applicable only to the young of some of the domestic animals. In several dogs three to twelve months old only a vestige of the round ligament could be found in the youngest animals and the suspensory ligament was already reduced to a very small triangular fold which represented approximately but 2 per cent of its original extent. With some slight qualifications this statement also applies to sheep, and to a somewhat less extent also to cats, guinea pigs and rabbits. In most of these animals the suspensory ligament at the time of birth usually extends, of course, along the ventral abdominal wall as far as the umbilicus and contains the umbilical vein in its free margin.

As has been stated elsewhere by the writer, the intra-abdominal portion of the umbilical vein in the sheep cannot and does not retract at time of birth, but remains filled with unclotted blood save in its *contracted* portion in the immediate vicinity of the umbilicus where it contains a small clot. But soon after birth the umbilical vein is freed from its attachment to the abdominal wall as a result of degenerative changes and then undoubtedly undergoes a delayed though a more rapid retraction and regression after the manner described by Robin ('60), Haberda ('96) and Baumgarten ('77) in the case of the vein, and as is true especially in case of the arteries in man. That this retraction and regression takes place comparatively rapidly is well shown by the fact that it had resulted in almost complete disappearance of the umbilical vein or so-called round ligament, in lambs 5 to 7 weeks old. Since degeneration of the round and suspensory ligaments are closely associated the condition of both these structures as found in this lamb and in dogs will be described with some detail.

Instead of a continuous suspensory ligament extending from the umbilicus to the region of the coronary ligament only a fine thread-like strand of peritoneum about 1 mm. wide extended from about the midpoint of the ventral surface of the liver to the abdominal wall about 4 cm. cranial to the umbilicus. Between the latter point and the umbilicus there was a small fold of peritoneum. The peritoneum in this region covered a small quantity of extra-peritoneal fat and was somewhat wrinkled but entirely smooth within a distance of 2 cm. of the umbilicus. Along the abdominal wall cranial to this strand and on the diaphragm, a fine, free fringe of peritoneum which become gradually wider could be seen very plainly and finally ran into a small triangular suspensory ligament from the dorsal extremity of the caudal margin of which another fold of peritoneum about half a centimeter wide extended to the central end of the above-mentioned fine strand. Between the latter and the suspensory ligament there was a large oval opening bounded by the ventral abdominal wall, the diaphragm, suspensory ligament, the liver and the above-mentioned narrow strand. From the dorsal or hepatic attachment of the latter a narrow fringe of peritoneum also extended caudally along the ventral surface of the liver to a small funnel-shaped fossa, or pit in the substance of the liver from the bottom of which projected a small, pointed, conical teat about one centimeter long and three millimeters wide at the base. Because of its appearance and location, this at once suggested the remnant of the umbilical vein. That it was actually such was confirmed later by gross and microscopic examination and it was interesting that this remnant still had a small conical lumen which was in communication with the left portal vein.

The suspensory ligament measured only 2.5 cm. along the hepatic surface and along the diaphragm and had a free crescentic border about 3 cm. long. It is evident, of course, that it and the fine strand as well as the narrow fringes represented only small remnants of the original extensive structure which had undergone almost complete degeneration so early in the life of the animal. Hence it is clear that we have here a very interesting and instructive stage in the degeneration and obliteration of

both the umbilical vein and the suspensory ligament which would undoubtedly have been complete a few weeks later. Consequently then, since the only remnant of the suspensory ligament in young sheep is a very small triangular fold and since even this small remnant disappears practically entirely in older animals it is evident that neither it nor the round ligament can be said to exist in adult sheep for both disappear completely during the first months of life. Evidently then the umbilical vein *never becomes ligamentous* although fibrous transformation may begin, but retracts and degenerates completely and the suspensory ligament becomes fenestrated and undergoes practically total destruction. It is apparent and interesting, however, that by far the greater portion of the suspensory ligament disappears very rapidly and that a very small triangular cranial portion may persist much longer or even permanently.

What is true of the sheep, also holds for the dog for in no case could a trace of either of these ligaments be found in mature or old animals. Moreover, in a dog approximately a year old there was not even an indication of the above-described teat-like remnant of the umbilical vein attached to the left portal as found in the sheep, and the suspensory ligament had already been reduced to a very small triangular structure only about 1 cm. broad which again represented only the most cranio-dorsal portion of the fetal structure.

Likewise in dogs approximately two months old there was only a vestige of the umbilical vein in the form of a short tag and only a small triangular remnant of the suspensory ligament. The latter measured 1.3 cm. along the diaphragmatic surface, 1.1 cm. on the hepatic and 9 cm. along its free caudal border. Hence the original ligament can be said to have degenerated almost completely. There was no round ligament whatever and the umbilical vein had retracted and degenerated so completely that nothing remained of it save a small teat or process 3.5 mm. long and 1.5 mm. thick which projected from the wall of the left portal vein exactly in the position in which the similar remnant had been found in the lamb. Hence it is evident that the processes of obliteration of the round and suspensory liga-

ments in the dog are wholly comparable to those in the sheep and that they occur with approximately equal rapidity.

A similar condition was also found in a dog about four months old. In this animal the one remnant of the umbilical vein was a fine strand about 5 mm. long and 1 to 1.5 mm. thick which was attached to and lay on the ventral wall of the portal vein at its bifurcation. The suspensory ligament too was somewhat smaller than in the above animal. Moreover, on one of several dogs only seven weeks old both the suspensory ligament and the umbilical vein had completely disappeared save for a very fine filament of peritoneum which extended from a point opposite the tip of the ensiform cartilage to the left portal vein. At the proximal or hepatic, end of this filament a small, short remnant of the umbilical vein was still present. In the other dog only a small filament 2.5 cm. long which was partly fibrous, was still attached to the left portal, and the suspensory ligament had completely disappeared save for a fine strand of peritoneum which was suspended from the ventral abdominal wall near the tip of the xiphoid process. However, that considerable variation exists in the time of the disappearance of the suspensory ligament, at least, is shown by the fact that in two dogs about one and one-half years old the suspensory ligament which was still present, began at a point opposite the middle of the xiphoid process. Yet the umbilical vein had wholly disappeared in both these animals. The findings in two pups three weeks old and especially in two new-born dogs are in marked contrast to those in the last two animals. In these four pups the suspensory ligaments had completely disappeared except for a very small falciform portion directly ventral to the vena cava. In both the new-born animals the umbilical veins ran directly from the left portal to the umbilicus and was completely isolated. Hence it is evident that whenever this condition exists the formation of a round ligament in the caudal border of the suspensory ligament had never included the vein or had already degenerated before birth, the latter was isolated and hence could no more form a ligament after becoming detached from the umbilicus than the omphalomesenteric or hypogastric vessels can do so after

these have become detached, retracted and degenerated, unless a permanent instead of a temporary attachment were subsequently acquired. Moreover, from the findings in these two new-born dogs it seems not at all unlikely that the two pups of the seventh week in whom no remnant of the suspensory ligament could be found were also instances in which degeneration and regression of the suspensory ligament had already begun at the time of birth.

Even in a pup 91 hours old marked changes were plainly evident in the distal portion of the umbilical vein as shown in



Fig. 1 Degenerating umbilical vein of a lamb 91 hours old. $\times 142$.

Fig. 2 Plicated caudal border of the suspensory ligament of a rabbit. In some portions these folds are fused. $\times 275$.

figure 1. In this case portions of the lumen of the vessel are filled with connective tissue containing some bloodvessels. The degenerating musculature which has undergone embryonic regression has lost its characteristic arrangement and staining qualities and also contains numerous blood vessels but can still be recognized as such. This vessel and these changes will be discussed fully below.

The degeneration and disappearance of the suspensory ligament and the umbilical vein in the cat occur much slower than in the dog and sheep. In the oldest cats examined no remnant

of either structure could be found, however, and in cats one-half to a year old, and occasionally in young kittens, it was not uncommon to find the suspensory ligament more or less fenestrated, a fact which can undoubtedly be correlated with its degeneration or disappearance. Not uncommonly the free caudal border of the suspensory ligament was rolled up as a scroll suggesting the presence of an unusually large round ligament but upon closer microscopic and macroscopic examination not a remnant of the vein nor a fibrous substitute for it could be found and the thickened border of the suspensory ligament was composed of folds of peritoneum or of loose connective tissue. This rolled up or pleated condition of the free caudal border occasionally seen was especially illustrated in the case of some rabbits and guinea-pigs as shown in figure 2.

In several rabbits over a year old from a third to a half of the suspensory ligament was still preserved although no remnant of the round ligament could be found, but in a guinea-pig of the same age the suspensory ligament was completely preserved from a point opposite the xiphoid process, although the round ligament could not be detected beyond the ventral surface of the liver.

That the rolled up or plicated border of the suspensory ligament can easily simulate the round ligament is excellently illustrated by the behavior of a special fold of the suspensory ligament which is occasionally reflected towards the gall bladder, to the right of the main ligament and which not infrequently contains a vein (fig. 3). When such is the case this reflection has a free border exactly similar to that of the main ligament and if one were to judge from gross appearance one would be compelled to conclude that there are two instead of but one round ligament in these cases for this vein is sometimes very thick-walled as in this instance. In rabbits the condition of both the round and suspensory ligaments is very similar. In guinea pigs they persist relatively longer.

Because of the great difficulty in obtaining cats, rabbits and guinea-pigs of definitely known ages the observations on them have been much fewer and hence any conclusions drawn from

them might require modification as a result of a more comprehensive series of observations. There is no question, however, that in all these animals the umbilical vein ultimately disappears first near the umbilicus, i.e., centripetally and not centrifugally as Robin and Herzog stated it does in man. It is also clear that the reason that the round ligament in most of the domestic animals usually seems to begin at the tip of the xiphoid is that the vein has retracted and degenerated and is forced against the ventral abdominal wall between the umbilicus and xiphoid by

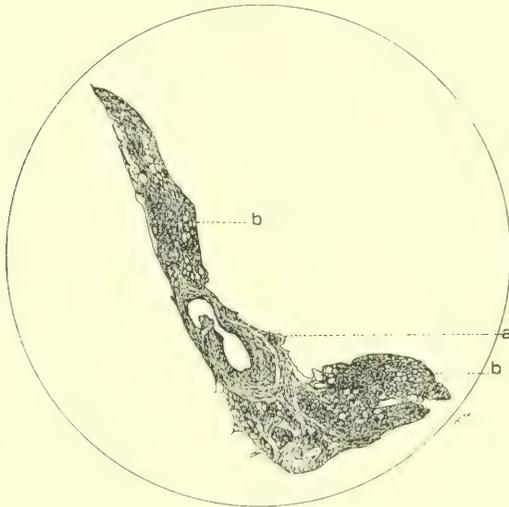


Fig. 3 Drawing of the unrolled border of an accessory free fold from the suspensory ligament to the gall bladder of the dog. The large thick-walled vein is shown at a point where one of its branches joins. *a*, vein; *b*, fat. $\times 42$.

the narrow regressing suspensory ligament in whose crescentic border it comes to lie, although in doing so it manifestly must take a more roundabout course than before. This is, no doubt, due to the fact that after its initial retraction the vein which is enclosed in the caudal border of the suspensory ligament is drawn cranially as a result of pre- or post-natal changes. Moreover, it is not at all unlikely that as a result of these factors the free caudal border of the ligament also becomes markedly concave

instead of remaining straight or approximately so. The entire absence of a fibrous substitute for the umbilical vein in the sheep, dog, etc., between the umbilicus and the xiphoid process must, it seems to me, be due to the retraction of the vein and would seem to suggest very strongly that Haberda is mistaken when he says that the amount of retraction determines the length of the fibrous filament. Moreover, it is not at all improbable that the tension exerted by the retracting and degenerating suspensory ligament is one of the factors responsible for the late retraction of the umbilical vein for it is scarcely conceivable that the contractile power of the degenerating musculature of the vessel would itself be sufficient to bring about this late retraction. Indeed, the absence of the round ligament or umbilical vein in the region between the umbilicus and the tip of the xiphoid process must be due to a comparatively rapid retraction and degeneration of the distal portion of the vein and it is especially significant that no fibrous remnant can as a rule be found between the umbilicus and the caudal extremity of the umbilical vein of animals, which is at all comparable to the fibrous remnants found between the umbilicus and the distal extremities of the hypogastric arteries in man. According to Robin, the adventitia of the arteries remains behind during the process of the delayed retraction in man and if it were also left behind in the later delayed retraction in non-ruminants one could suppose that the fine, fibrous, filaments between the hypogastric arteries and the umbilicus, in man, might have such an origin, but as will be seen later, such an assumption is unnecessary.

The rate of degeneration of the suspensory ligament and especially of the umbilical vein, in cats seems to be determined very largely by the previous existence or by the genesis of venous radicles which pour their blood into its caudal extremity. Such a condition was noticed very commonly in cats and guinea-pigs but never in dogs and sheep, although the degenerating umbilical vein may rarely acquire a secondary attachment in these animals also. It is a rather remarkable thing that after becoming detached at the umbilicus, the retracted umbilical vein not

infrequently receives several exceedingly minute venous radicles which lie immediately extra-peritoneally at the ventral surface of the thick fold of fat constantly present in cats between the umbilicus and a point opposite the base of the xiphoid process. These venous radicles become gradually larger proximally and can sometimes be seen to unite and to join the tapering extremity of the unpaired retracted umbilical vein at a point opposite the middle of the xiphoid process. This peculiar relationship was occasionally very evident because all the vessels beginning with the finest veins even, were very full of blood and the umbilical vein could be seen without the least difficulty to empty into the left portal. It might at first thought seem probable that this main vein is a para-umbilical or Burow's vein instead of the true umbilical vein, but microscopic as well as gross examination shows this not to be the case. Moreover, it would be remarkable that all trace of the umbilical vein should have disappeared in a *kitten* but ten weeks old, for example. This transformation of the major portions of the umbilical veins into an integral, even if but a temporary, part of the peripheral venous system is particularly significant in its bearing upon the degeneration and disappearance of the vein and the subsequent formation of a round ligament. Occasionally also a large lymphatic trunk (figs. 4 and 5) several millimeters in caliber runs parallel and adjacent to the vein in the caudal border of the suspensory ligament directly cranial to the vein. The unusual size, distension and *beauty* of this trunk which joins the larger lymphatic vessels at the hilus of the liver makes it very conspicuous and it is evident that its presence and that of a converted and actively functioning umbilical vein must have a very important bearing on the time of disappearance of the vein and of the suspensory ligament. That the lumen of the umbilical vein may be preserved longer where branches are located was well established by Baumgarten and by Kirchbach ('99) for man.

In case of the kitten two weeks old the umbilical vein had completely lost its characteristic walls although the lumen was still about .75 mm. in size and full of blood. The walls were composed only of an endothelial lining bounded by connective

tissue and there was an entire absence of non-striated muscle even within a half centimeter of the left portal. Nevertheless, that this vein was the original umbilical vein is shown by the entire absence of any remnant or vein in the caudal border of the suspensory ligament. Moreover, the total disappearance of the umbilical vein at so early a day in cats is, of course, very unlikely.

Apparently then, in these animals the retraction and degeneration of the suspensory ligament takes place *pari passu*

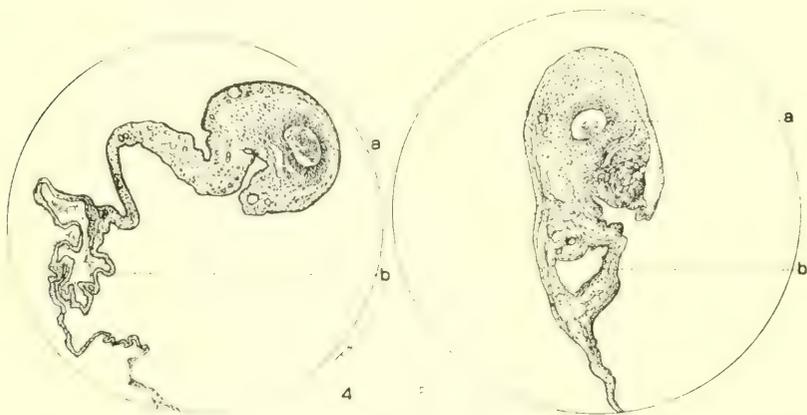


Fig. 4 Round and suspensory ligaments of the cat. *a*, remnant of the umbilical vein; *b*, a large lymphatic. $\times 97$.

Fig. 5 A section of the suspensory and round ligaments of the cat. *a*, remnant of the umbilical vein; *b*, lymphatic. $\times 79$.

with that of the umbilical vein and is dependent upon and determined by the retrogressive changes in the vein to a certain extent at least. That these processes are more or less independent of each other, however, is well illustrated by two dogs over a year old in which the suspensory ligaments were well-preserved while the umbilical vein had disappeared completely. A similar condition is also not uncommonly found in old rabbits, cats and guinea-pigs. On the other hand, in the dog and sheep in which the umbilical vein becomes totally isolated and never has any permanent connections with the peripheral veins it de-

generates very rapidly. But in the cat, guinea-pig, rat and rabbit in which the vein is not isolated and in which the suspensory ligament degenerates much slower, it occupies a relation similar to that of the hypogastric arteries in the same animals. In these animals it is also exceedingly common to find one or more small veins running roughly parallel to and in the immediate vicinity of the degenerating umbilical vein. The largest of these is usually plainly visible to the naked eye but the rest are generally of microscopic size only. Although injections of these vessels were not made yet from observation with the unaided eye and from a study of sections of the ligaments they apparently correspond to the para-umbilical veins of Sappey rather than to the vein of Burow. The largest of these veins which does not join the degenerating umbilical vein is generally plainly visible because it is filled with blood. In the dog there frequently are also a large number of microscopic veins in the adventitia of the degenerating umbilical vein which join the latter. These seem to be most numerous at the distal extremity of the vessel where the lumen is frequently multiple, and arise from the vasa vasorum.

The behavior of the hypogastric arteries in these animals was quite different from that of the vein for they retract quite early and usually become attached to the apex of the bladder. They had already retracted in young rats whose eyes had not yet opened and could be traced peripherally only as far as the apex of the bladder. In a rat approximately three months old no trace of them could be found microscopically in this region. In cats eleven to twelve days old they have usually begun to retract although the urachus is often and the umbilical vein is always, still attached to the umbilicus at this time. It is also true, no doubt, that the time of separation of the arteries is dependent upon the time of sloughing of the cord, which undoubtedly varies as much in animals as Weckerling's ('08) extensive analysis showed it does in the human infant. In a dog one week old the vessels were still firmly attached and there was no indication of a beginning retraction. These observations confirm those of Robin although I cannot corroborate his observation that the retraction of the arteries is always simul-

taneous. This is perhaps never the case if one of them becomes infected, for the infected vessel remains attached longer. But even excluding infections it is not at all likely that the two arteries always rupture simultaneously.

In a few instances in cats it was also noticed that the retracting arteries and urachus drew the vein caudally across the internal surface of the umbilicus. Such occurrences were well described and illustrated in Robin's excellent investigation.

Robin stated that the obliterated hypogastric arteries usually do not remain attached to the summit of the bladder in ruminants and carnivora. As far as sheep are concerned, Robin's statement is confirmed, but in dogs and cats they are almost always attached to the urachus or to a cicatricial formation at the very apex of the bladder. The same statement holds for young rats, guinea-pigs and rabbits although the minute size of the vessels in these animals makes it much more difficult to observe and trace them with the unaided eye. Besides, it is not rare to find the distal ends of the vessels more or less coiled or tortuous shortly after they become detached from the umbilicus or even much later in the sheep. Hence it seems strange that a secondary attachment is acquired to the apex of the bladder. This attachment which is a fibrous one is no doubt due to the fact that before the vessels become detached, the bladder and urachus occupy a position between the converging hypogastric arteries. Hence as the bladder becomes distended they are forced firmly against its sides and may even infold them. After becoming detached the atrophy or retraction of the lateral folds of the peritoneum in which the arteries lie draws them or at least holds them, in intimate contact with the lateral walls and the conical apex of the bladder and the degenerating urachus. Were it not for this fact it would seem to follow that the detached free ends of the hypogastric arteries would be retracted passively more and more with each successive dilatation of the bladder until they reached a point far from the apex. After they become attached to the latter further retraction is, of course, impossible and the further obliteration or disappearance of these vessels must hence be due to atrophy, degeneration

and to a fibrous transformation *in loco*. Since, in contrast with the vein the atrophic changes in the arteries take place very gradually it is apparent that the condition in which the vessels are found depends much upon the age of the animal. In old dogs, for example, the degenerated and transformed arteries usually cannot be seen near the apex of the bladder unless the latter is distended and then only as very fine, fibrous, often more or less discontinuous cords which gradually become somewhat thicker as the base of the bladder is approached. As already stated, instead of being attached to the apex of the bladder the free ends of the retracted hypogastric arteries in the lamb usually lie more or less curled up in a wide and loose fold of peritoneum at the sides of the bladder just lateral to its apex. This position is probably very largely due to the fact that they retract actively immediately after birth and not simultaneously with the urachus. Moreover, they lie in broad, loose peritoneal folds instead of being intimately associated with the bladder and urachus and with each other for ten or twelve days or more, before retraction can occur and this later retraction is a very gradual and not a sudden and extensive one.

The fact that the arteries which retract earlier degenerate much later than the vein, especially in the dog and sheep, has already been mentioned and some of the factors involved have been suggested. A further factor it seems to me lies in the fact that after becoming attached to the urachus and the apex of the bladder the arteries are alternately stretched and relaxed with each successive distension and evacuation of the bladder. While this stretching is a purely passive process the relaxation may nevertheless be accompanied by a certain amount of active contraction of the vascular musculature the effect of which may be retarded atrophy and degeneration.

In rabbits twelve days old the hypogastric arteries and urachus had already retracted completely and their free ends met at the apex of the bladder while in a rabbit one year old they could not be detected except in the region at the base of the bladder because they had atrophied so completely.

In several cats one year old they were still well-preserved and firmly adherent to the apex of the bladder but in two animals only three months old they could, on the contrary, scarcely be detected and could be traced only a little beyond the base of the bladder. Often, however, when the atropic fibrous substitute is invisible in the contracted state of the bladder it can easily be detected upon distension and it is evident that their presence or absence in the lateral surfaces and the apex of the bladder is probably largely if not wholly dependent upon the fact as to whether or not they gain a secondary attachment to the degenerating urachus.

Since the sudden marked retraction which occurs in the hypogastric arteries of the lamb can not occur at all in the dog, cat, rabbit and guinea pig in which only a gradual retraction takes place some days or weeks after birth, it would be possible to construct a series beginning with man, in whom retraction is evidently slight, inconstant and always delayed and ending with the sheep and other ruminants in which it is immediate and practically complete a few hours after birth. It has seemed to me that as far as man is concerned the slight amount of the late retraction and the slowness of it, may be due in part at least to the somewhat different relations of the arteries and the bladder to the abdominal wall and peritoneum. In the domestic animals the urinary bladder is practically an intra-peritoneal organ while in man it is extra-peritoneal. Hence in man the hypogastric arteries lie between the peritoneum and the transversalis fascia for a comparatively long distance. Moreover, because of the different position which it occupies in man, evacuation of the distended bladder cannot assist much in the retractions of the vessels in the infant. Moreover, the descent of the bladder from the region of the umbilicus is much more gradual even if finally more pronounced when the adult condition is reached. It is of doubtful value and validity, to be sure, to compare post-natal degenerative processes in animals born in such widely varying states of maturity, which have such varying life cycles and which grow at still more widely varying rates, nevertheless the fact remains that in spite of these differences very similar

processes occur at somewhat different times in all animals. The differences that exist are of degree rather than of kind.

It is not at all uncommon to find a small amount of blood near the free ends of the torn hypogastric arteries in the lamb but such was, of course, never the case in animals in which the vessels are ruptured extra-abdominally and in which only a delayed retraction occurs. The presence of extensive clots within the vessels was rare in the lamb but common in the other animals for in them an effective obliteration of the lumina was probably made difficult by the fact that the vessels remained attached to the umbilicus thus making contraction of the intra-abdominal portions more difficult. Since the presence of clotted or unclotted blood must of necessity, prevent rapid obliteration of the lumen of a vessel and delay retrogressive changes in it it does not seem probable that the presence of clots could aid much in preventing hemorrhage as Gmelin thought.

Since the ends of the retracted hypogastric arteries in lambs, often projected freely into the peritoneal cavity rupture of the peritoneum must, of course, have taken place. Since the evacuation of the bladder takes place at intervals before these vessels become detached without producing this lesion and since the end of only one of the retracted vessels may project intra-peritoneally in the newborn lamb, it does not seem likely that the combined force resulting from the retraction of the detached arteries and the contracting bladder is sufficient to produce a rupture of the overlying peritoneum. It is true that in the lamb the immediate, sudden elastic recoil of the arteries may be associated with or even stimulate, the evacuation of the bladder which usually occurs soon after or even during birth, but these combined forces could scarcely rupture the peritoneum. Hence it seems quite obvious that the latter is torn as a result of the outward traction produced at the time of rupture of the cord. This conception is also in accord with the statement of Hemborg that the arteries tear intra-abdominally although as previously stated I am inclined to believe that rupture in ruminants occurs extra-abdominally but in portions of the vessels which previous to traction and rupture were intra-abdominal.

Since the observations here reported were observed largely incidentally no comprehensive detailed microscopic study of the minute changes which accompany the processes of retraction, atrophy and degeneration of the umbilical vein and hypogastric arteries were made. However, quite a number of specimens from several species were examined and from these it is evident that the microscopical picture of the so-called obliteration of the umbilical vessels in the domestic animals, save rarely, is not practically concluded a few weeks after birth as Kirchbach asserts is true of the ductus venosus and vena umbilicalis in man. Kirchbach's statement is contradicted also by Haberda. Attention has already been called to the fact that, as a rule, the umbilical vein in both the dog and sheep disappears totally in the course of a few months. From examination of the remnants of the veins found before this time and from other facts stated above it is obvious that in the animals examined, this obliteration, atrophy, degeneration and absorption is invariably centripetal and not centrifugal as Robin says is the case in man. The free distal extremities of the filaments representing the remnants of the umbilical vein which were still attached to the left portal, were composed of vascular ill-preserved, fibrous connective tissue enclosed in peritoneum except in case of one lamb in which bundles of muscle fibers were preserved even in the degenerating tip. Farther proximally, i.e. nearer the left portal, a small remnant of the umbilical vein remained, but still farther centrally a remnant of the lumen was also present, and finally a portion of the original vessel with well-preserved walls somewhat reduced in size could be recognized. In case of the lamb about 5 to 7 weeks old the small degenerating remnant of the vein which was approximately 2 cm. long contained a macroscopic lumen for about half its extent but the intima was poorly preserved and absent in places. There was no proliferation of the endothelium and an elastica interna was not noticed in Van Gieson stains. The media which had undergone a hyaline degeneration, was represented only by a granular detritus in some places and small degenerating nuclei were accumulated in it near the lumen. There was no distinct

adventitia and the fibrous connective tissue which surrounded and invaded the irregular degenerating mass also seemed to be in process of degeneration.

The distal ends of the veins in dogs and cats were practically in the same condition although in some cases the original lumen had become multiple by being encroached upon by the folded degenerating media. It not infrequently contained some erythrocytes in a fair state of preservation and occasionally the adventitia was very vascular where it was well-preserved. Numerous small vessels were also found in the degenerating media and not infrequently these communicated with the original lumen to which they ran more or less parallel. No cellular infiltration such as described by Haberda for man, was seen and the whole picture was that of a degeneration rather than that of an obliterative endarteritis. In fact, aside from the presence of the small blood vessels in the connective tissue the impression is usually that of a passive rather than of an active process and it is difficult to see why a purely temporary complete substitution of fibrous connective tissue should occur in the veins only to be absorbed as soon as formed. Such a complete substitution of fibrous tissue for the vein may occur, to be sure, in animals in which a true ligamentum teres hepatis is formed, but not, of course, in those in which it never comes into existence because of early resorption of the vein. Nevertheless, that a partial transformation may take place even in these animals has already been stated regarding the umbilical vein from a dog 91 hours old a portion of which is represented under higher magnification (figs. 6 and 7). Although this animal was less than four days old the lumen of the peripheral portion of the vein which was still attached to the umbilicus, is completely obliterated by a slightly vascular connective tissue and the degenerating media has become vascularized. The muscle fibers have lost their outlines and specific staining reactions and are represented by a degenerating mass containing irregular nuclei. Somewhat farther centrally, i.e., nearer the liver, this vascularization is much more evident as shown in figure 7 and the fibrous connective tissue filling the lumen has more the appearance of newly-

formed tissue. Still farther centrally the regular, reduced lumen which contains blood is surrounded by a well-preserved intima and a better preserved though degenerating musculature (fig. 8). In the case of this dog then the peripheral portion of the lumen of the vein became completely obliterated through connective tissue formation while its musculature was degenerating and being penetrated by numerous vessels arising from the vasa vasorum, in spite of the fact that the vessel was to undergo rapid degeneration and absorption.

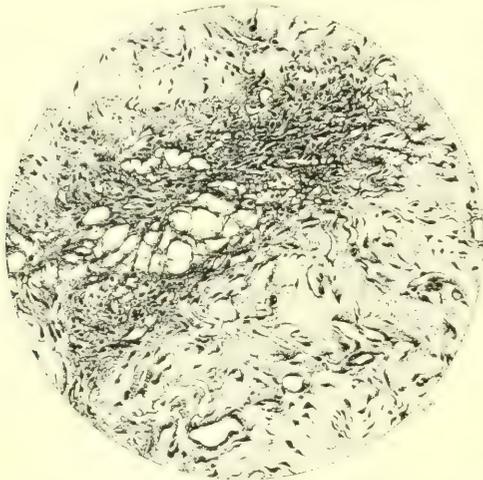


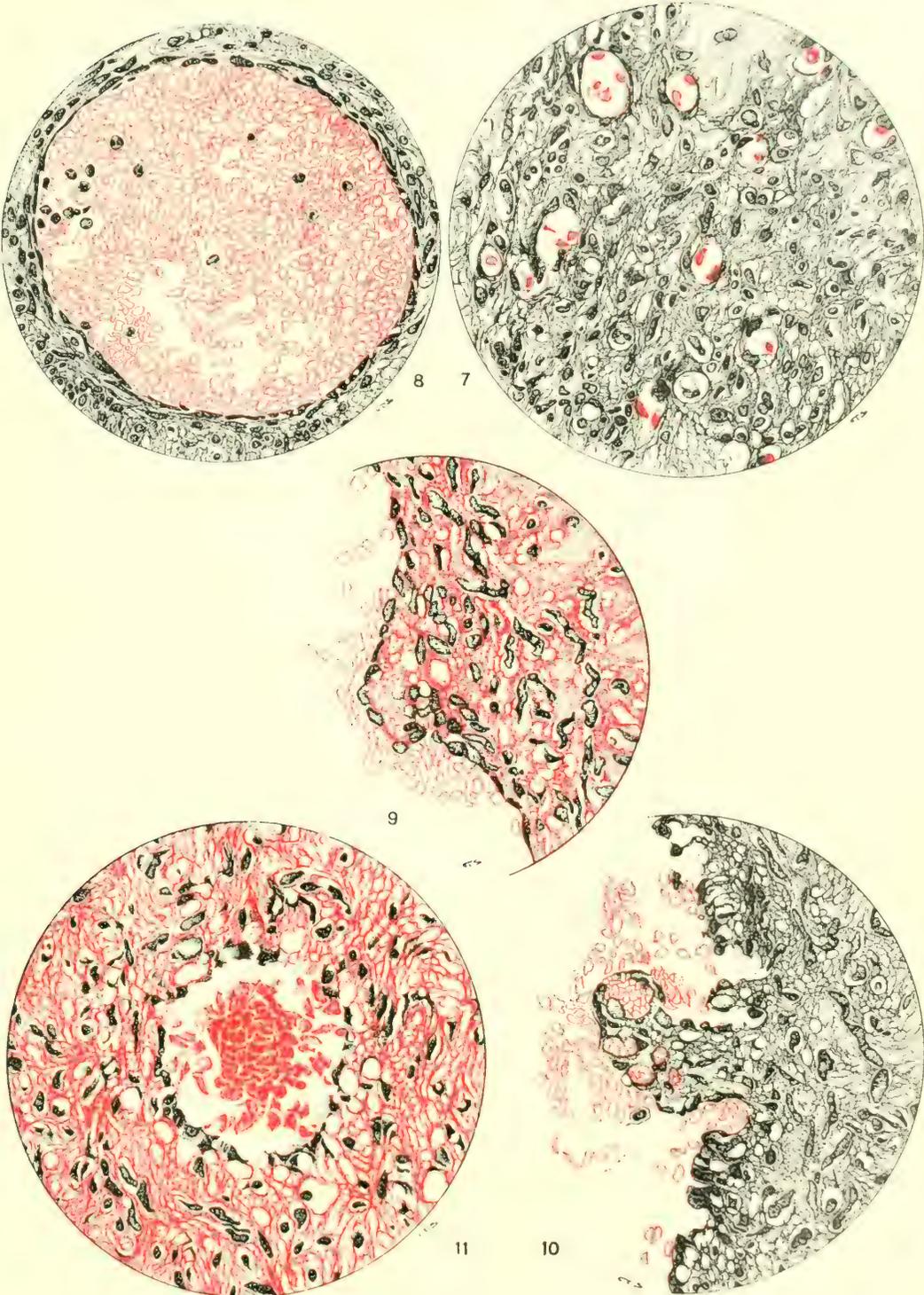
Fig. 6 Central portions of umbilical vein of a dog 91 hours old, showing the lumen obliterated by vascular connective tissue surrounded by the degenerating media. $\times 275$.

The presence of such profound changes so soon *post natum* confirms the observation that embryonic regression of the umbilical vein sometimes begins before birth. In several instances it was noticed, for example, that the cross-section of the media of the umbilical vein lacked all the characteristics of plain muscle and looked not unlike a synecytium. Such appearance could, of course, be explained only by prenatal degenerative changes or by the supposition that the musculature of the wall of the vein sometimes fails to reach maturity during fetal life—a

PLATE 1

EXPLANATION OF FIGURES

- 7 A different portion of the obliterated vein shown in figure 6. $\times 630$.
- 8 Umbilical vein of the dog; same as figures 6 and 7 but somewhat farther centrally. $\times 450$.
- 9 Portion of wall of umbilical vein of dog 45 hours old, showing process of degenerating media extending into the lumen. $\times 750$.
- 10 From the same vessel as figure 9, showing process formation, vacuolation beneath the endothelium and vascularization of the process. $\times 750$.
- 11 Omphalomesenteric vessel from a dog 44 hours old, showing degenerating endothelium and media and some ill-preserved blood in the lumen. $\times 750$.



supposition which would, however, fail to explain the early fibrous transformation above mentioned. Moreover, the surprising fluctuations in the duration of pregnancy which have been observed would seem to make the occurrence of pre-natal degenerative changes not impossible.

In another dog but 45 hours old the conditions were quite different for the lumen of the vein which was patent contained uncoagulated blood except in the immediate neighborhood of the umbilicus. Nevertheless, degenerative changes had already appeared and were particularly evident in the musculature especially immediately beneath, i.e., external to the endothelium. The cell outlines were lost and the media was represented by a uniformly ill-preserved syncytial mass which easily took an acidophile stain. The nuclei were very large, vesicular, and irregular in outline and arrangement. The intima was quite well preserved in most places but in others the cells which were irregular in form had become elongated, the nuclei were large and swollen and large vasculoles were found between and especially below the cells of the intima. The most striking thing, however, is the occurrence of projections of club-shaped processes into the lumen as represented in figures 9 and 10. In the case of figure 9 this projection was still capped by the degenerating endothelial cells on which erythrocytes lay. The same is true of the similar though larger and longer projection represented in figure 10 which is taken from the same vessel somewhat farther peripherally. In this case the elongation and vacuolation of the endothelial cells some of which have been cast off, are well seen but the honey-combed process is still capped by some of them.

Since no series of vessels taken from pups only several hours apart in age were examined it is impossible to say whether this process formation is a constant phenomenon in the degenerating vein in the dog. It was not observed in the sheep and before discussing the significance of these facts I desire to call attention to changes observed in the hypogastric arteries and the omphalomesenteric veins. In case of two of the latter which were still attached to the abdominal wall in a dog 91 hours old similar

degeneration of the endothelium and the musculature was present but the formation of processes was not observed. As shown in figure 11 the intima which is absent in places, has undergone degeneration although the lumen still contains blood. Figure 12 shows a section of the accompanying omphalomesenteric vessel apparently in a more advanced stage of degeneration, for here the whole lumen is obliterated by the large cast-off endothelial cells and fibrin which form a network completely filling it and

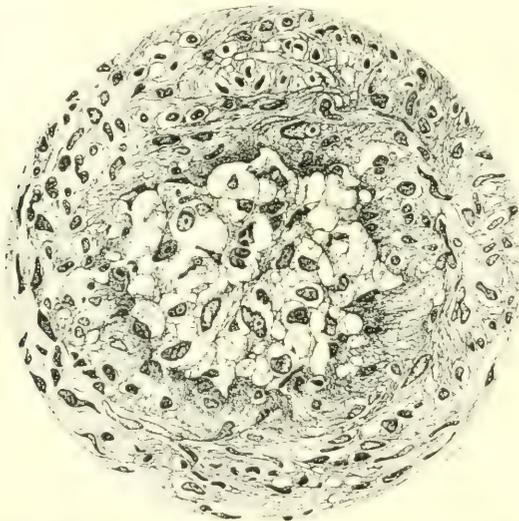


Fig. 12 Transverse section from an omphalomesenteric vessel of a dog 44 hours old. Lumen filled with desquamated endothelium and a fibrin network. $\times 750$.

simulating endothelial proliferation and migration. In the case of both these vessels the musculature has undergone considerable degeneration, and readily takes an acidophile or special connective stain, making it difficult to distinguish the connective tissue which is, of course, also a purely temporary constituent.

In case of the hypogastric artery of the same dog no process formation is present and the intima has completely disappeared over a considerable extent of the distal portion. The lumen which is not lined by endothelium although containing blood is

surrounded and encroached upon by a wide band of fibrous tissue in some of the large meshes of which isolated irregular degenerating nuclei lie. However, as shown in figure 13 there are no signs of endothelial proliferation or of infiltration and the outer layers of the media are quite well-preserved. At the extreme distal portion of the vessel all of the musculature is intact and the lumen has its characteristic shape. Slightly farther proximally it contains some blood, the intima is absent and where the blood lies in contact with the wall the meshwork of connective tissue extends out into it and contains newly formed vessels (fig. 14). The rest of the lumen is bounded by the circularis of the media for no *elastica interna* is present in this portion. Where the blood which is not formed into a thrombus, fills the whole lumen this proliferation of connective tissue is evident over the entire circumference and in some places the lumen is entirely pervaded by it. The relations of this connective tissue to that in the media are so clear that one cannot doubt that it is a direct continuation and remnant of that which was contained between the muscle bundles of the media and that some of the fenestra in the network previously contained muscle-fiber bundles which have degenerated and have been absorbed.

That these degenerative and obliterative changes are not invariable or constant in occurrence, however, is shown by a specimen of a hypogastric artery taken from a dog about a year and a half old as shown in figure 15. In this case all the constituents of the wall of the vessel including the endothelium and the *elastica interna* are still present and well-preserved although the connective tissue is greatly increased in amount especially at the periphery. The vessel is gradually becoming transformed by the proliferation of the adventitial and inter-fascicular connective tissue which is always present in comparatively large quantities in vessels from mature fetuses. Farther centrally (fig. 16) in the vessel—i.e., nearer its origin—there is but little connective tissue near the lumen which is surrounded by poorly preserved endothelium, a partial *elastica interna* and a very well-preserved media which on one side is encroached upon markedly and replaced by fibrous tissue.

The conditions as found in this dog are quite different from those found in the hypogastric arteries of a lamb three and one-half weeks old a section of which is represented in figure 17. In this case there are portions of the vessel in which the lumen is completely surrounded by a thin layer of fibrous tissue (*a*) which varies considerable in thickness but which lies internal to the elastica interna (*b*). Although the endothelium is absent in places the musculature is well-preserved where it is not invaded by the connective tissue, and contains many elastic fibers (*c*). In the more distal portion the vascular fibrous connective tissue plugs the whole lumen of the vessel and serial sections toward the tip show that it did not enter from without through the free extremity. The intima is preserved in some places and the connective tissue then lies between it and the elastica interna. Where the intima is absent it lies in long strands in the lumen. The connective tissue internal to the elastica is directly continuous in some places with that external to it which lies between the muscle bundles of the media. Near the extremity of the vessel the inner layers of the media are encroached upon and entirely replaced by the vascular connective tissue which plugs the entire lumen. Hence, I am inclined to believe that the process of obliteration in this lamb is entirely comparable to and represents a later stage than those found in the hypogastric arteries of the dog as represented in figures 13 and 14.

From these things it is evident that obliteration of the hypogastric arteries in the dog and sheep may take place in at least two ways. In one case the degenerating musculature is replaced by fibrous tissue which encroaches upon it and upon the lumen from the periphery toward the center and in the other the lumen is encroached upon and the surrounding musculature displaced by connective tissue arising from within after degeneration and desquamation of the intima and proliferation of vessels from the vasa vasorum. It is evident of course that whenever this transformation takes place from without, i.e., from the periphery, the lumen and its bounding intima as well as the inner layer of the media, may be preserved very much longer than in the

PLATE 2

EXPLANATION OF FIGURES

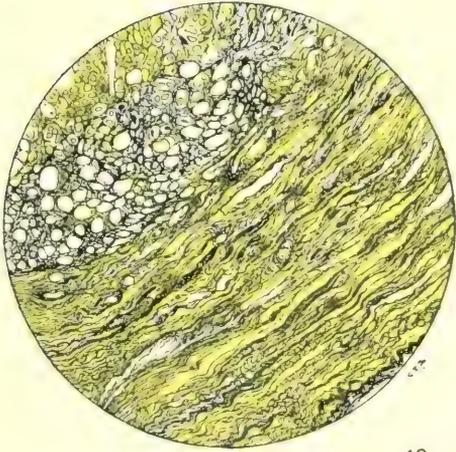
13 Inner layers of a hypogastric artery from a dog 91 hours old. $\times 410$. Van Gieson stain. Connective tissue, black; muscle and blood, yellow.

14 From a hypogastric artery from a dog 91 hours old, showing the obliteration of the lumen through vascular connective tissue. $\times 630$.

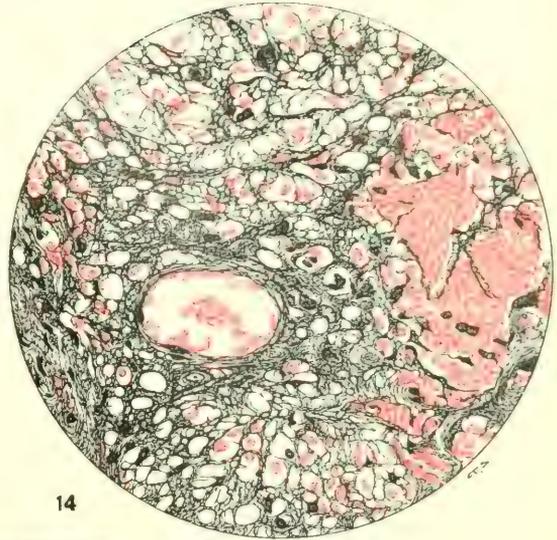
15 Portion of the wall of a hypogastric artery of a dog $1\frac{1}{2}$ years old. $\times 62$. Connective tissue, black; muscle, brown.

16 Portion of the vessel shown in figure 15 but somewhat more centrally. $\times 630$. Van Gieson stain. Connective tissue, black; muscle, yellow.

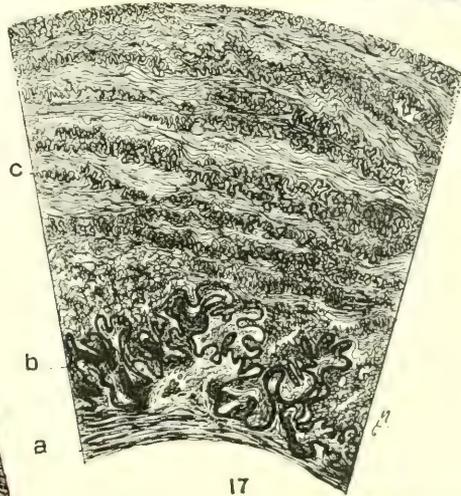
17 A section of the inner two-fifths of the wall of a hypogastric artery of a lamb $5\frac{1}{2}$ weeks old. Note the extremely folded elastica interna, the absence of an intima, and the presence of a layer of fibrous tissue between the latter and the elastica as well as numerous elastic fibers in the media. $\times 620$.



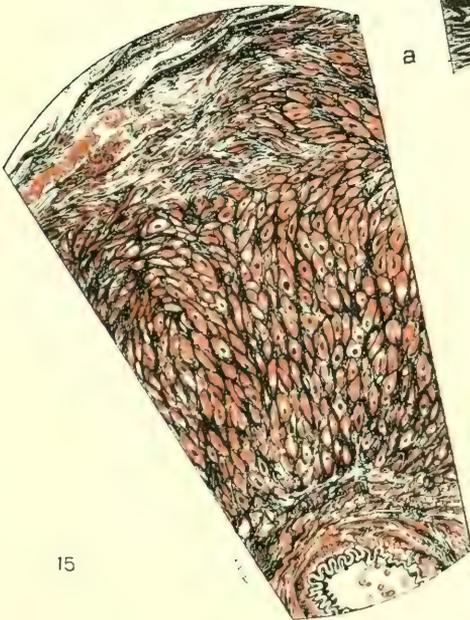
13



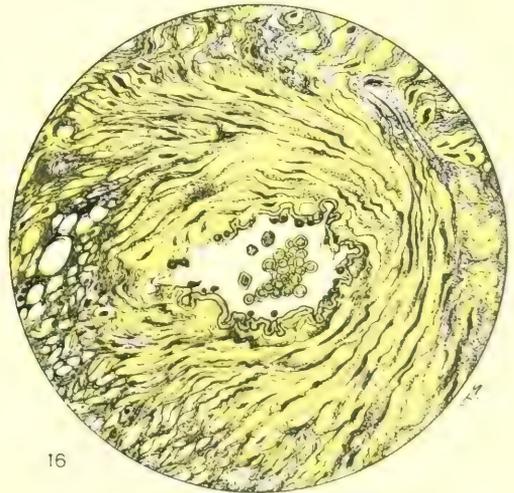
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17



15



16

other case and it is also evident that both processes of obliteration may be present at the same time.

From the facts observed especially in the umbilical veins of the dog and sheep, it is apparent that similar processes are initiated in them but that they cannot extend beyond the initial stage because of the rapid degeneration and absorption of these vessels. In the cat, rabbit and guinea-pig, on the other hand, in which the disappearance of the umbilical veins is a much slower process a corresponding and complete fibrous transformation may take place.

So far as the umbilical vein and hypogastric arteries of these animals are concerned no evidence whatever for the origin of connective tissue from endothelium has been obtained. Merkel also ('03) insists that the endothelial cells do not take part in the organization of a thrombus, and asserts that Baumgarten was mistaken when he concluded that an actual proliferation of the endothelial cells takes place. Nevertheless, in the absence of corroborative facts it is not denied that Haberda was mistaken when he declared that in man "Die our Obliteration der Nabelgefäße führenden Veränderungen bestehen in einer zu Bindegewebe sich metamorphosierenden Wucherung des Gefassendothels der sich an den Nabelenden der Gefäße eine von der Nabelwunde ausgehende entzündliche Zelleninfiltration hinzugesellt." This proliferation which is said to take its origin from the endothelium of the obliterating vessels was described by Thoma, confirmed by Baumgarten, emphasized by Hauptmann and in a measure corroborates the observations of Mall ('02 and '12) and of Kling ('02) to the effect that reticulums may arise from endothelium.

A very interesting phenomenon was noticed in the extra-abdominal portion of an umbilical vein of a lamb which died during labor. In the case of this lamb a portion of the vein contained blood which was in process of coagulation. Upon microscopical examination the lumen which was fairly regular save for small ridges was seen to be dilated and filled with blood containing strands of fibrin and a fibrin network in some places. The whole vessel was well-preserved, an elastica interna was present, and the endothelium was intact save in some places

where the cells had become detached or where fibrin strands were in contact with the wall. In these places the endothelial cells had wandered far out into the lumen along the strands of fibrin. In some places there were small accumulations of endothelial cells and not infrequently the presence of protoplasmic processes made them quite irregular in form. In places where a number of these cells were found in a fibrin network the appearance roughly simulated that of an embryonic connective tissue. These endothelial cells were all well-preserved and the only cells in process of degeneration were some of the blood cells contained in the clot. The musculature also was well-preserved except in two areas on approximately opposite sides of the lumen where the outlines of the inner fibers were obliterated and their place taken by irregular lumps of hyaline material which stained very intensely with hematoxyn and suggested degenerative changes. No evidences of mitoses were present in the endothelium and there was no cellular infiltration.

From what I have seen I am inclined to believe that the presence or absence of blood in the lumen of a vessel is of considerable importance, for in not a single instance were early obliterative processes observed in the empty contracted and retracted distal extremities of the hypogastric arteries. It is worth recalling in this connection that Haberda thought that the more rapid obliteration of the arteries is due to the fact that the thrombus in them is less extensive and that the walls of the vessels are in contact. There is considerable variation, however, in the rate of the complete contraction of the vessels for in a dog which died of inanition 44 hours after birth, the vein was practically empty and quite well contracted throughout its extent while the arteries were markedly distended with blood. But in another—a still-born pup from the same litter—the conditions were exactly the contrary. In the lambs examined the hypogastric arteries were always empty save for small clots or unclotted blood here and there. The vein, on the contrary, was always filled with uncoagulated blood except for the presence of a small clot in the beginning of its unpaired portion and my findings in this regard were entirely comparable to those of

Pöllot ('09) in the ductus arteriosus in which the retrogressive changes were usually not accompanied by thromoses.

Since the retrogressive changes in the umbilical vein of the cat, rat, rabbit and guinea-pig are so much slower and take place in direct relation to and not largely or wholly independent of those in the suspensory ligament as is the case in the dog, a somewhat different condition might be expected to exist. This is the case for in these animals the vein persists much longer throughout a portion of its extent, and not infrequently a fibrous structure which may somewhat rightly be termed a ligament is substituted for it (figs. 18, 19 and 20). However, as already stated, so far as the vein retracts or degenerates completely it is not replaced by a fibrous cord and hence the process of degeneration in this, the distal portions, is from all appearances identical, as a rule, with that in the dog and the sheep. In the more proximal portion of the umbilical vein a roughly cylindrical cord composed of loose vascular fibrous connective tissue of hyaline appearance which is sometimes markedly canalized is often found in cats, rabbits and guinea-pigs. In cats from eleven days to one year old this thickening in the caudal border of the suspensory ligament is usually due to the presence of a band of well-preserved fibrous connective tissue in whose proximal or intra-hepatic portion a vessel which can be recognized as remnant of the umbilical vein is usually present (figs. 4, 5, 18, 21). But in its distal—or hepatic—portion near the xiphoid process, no such remnant can be detected as a rule in cats only six months old and occasionally no trace whatever of the umbilical vein was found in any portions of the caudal border of the suspensory ligament examined in cats one year old (figs. 19, 20, 21, 22, 23). Indeed, in one instance such was the case in a cat only six weeks old and it is worthy of special notice in this connection that in the specimens from these and still younger cats, eleven days old, the picture was always that of a pure degeneration without the least indication of an endothelial proliferation and that in those instances where remnants of the lumen were found the endothelium was one-layered and well-preserved in places.

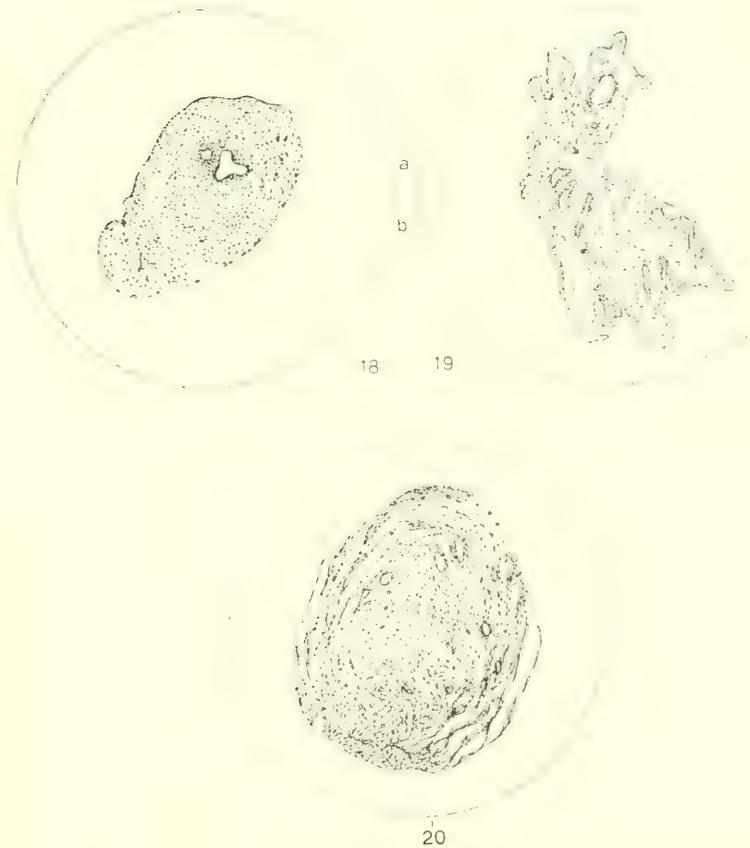


Fig. 18 Proximal portion of the degenerating umbilical vein from a kitten fourteen days old. With higher magnification the original lumen is clearly recognizable. *a*, remnant of original vein; *b*, connective tissue wall. $\times 142$.

Fig. 19 Transverse section of the free caudal border of the suspensory ligament of a cat about six weeks old. It contains no recognizable remnant of the umbilical vein, is quite vascular and composed of very loose fibrous connective tissue bounded by peritoneum. $\times 97$.

Fig. 20 Transverse section of the peripheral portion of what seemed to be a round ligament in a kitten seven days old. There is no trace of the umbilical vein in the very fibrous connective tissue. $\times 142$.

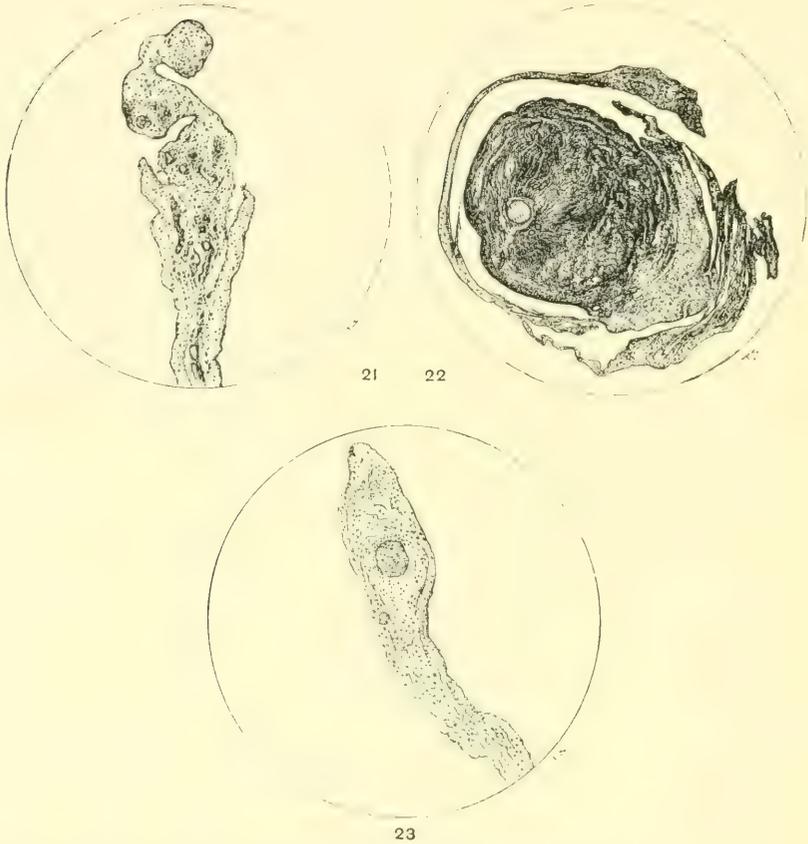


Fig. 21 Distal extremity of round ligament from a kitten 24 days old. No remnant of the umbilical vein is present in the vascular connective tissue. $\times 142$.

Fig. 22 Round ligament of a kitten 14 to 18 days old. No recognizable remnant of the umbilical vein is present. The ligament is composed of a very loose connective tissue not unlike embryonic connective tissue which looks gelatinous. $\times 54$.

Fig. 23 Transverse section from the free caudal border of suspensory ligament of the cat, containing a large vein with nothing but an endothelial wall bounded by connective tissue. This vein bears no resemblance to the remnant of the umbilical vein. $\times 142$.

The fibrous connective tissue which had replaced or encroached upon the vein varied from a poorly preserved dense fibrous connective tissue to a very loose embryonic tissue which because of its gelatinous appearance suggested in the gross even the umbilical cord to a laboratory assistant (figs. 19, 20, 22). It seldom contained any recognizable remnant of the lumen of the original vein as stated by Kirehbach for the round ligament of man. When the original lumen was preserved it had always become much reduced and often multiple, as a result of folding, as described by Baumgarten in man. In some cases the tissue surrounding the remnant of the vessel was very loose and vascular and the irregular outlines of the periphery of the wall of the degenerating veins generally suggested that it was enclosing the vein rather than that newly-formed elements of intimal origin were actively transforming the vessel from within. Moreover, the inner portion of the media was often better preserved and the lumen of the vessel often contained some erythrocytes while the outer portions of the media, on the contrary, were degenerated. However had this process of degeneration been observed at closer intervals in stages of a few days, e.g., it is not at all unlikely that a certain amount of cellular infiltration, as described by Baumgarten and Haberda in the neighborhood of the umbilicus in man, might have been noticed in cats as well.

The most striking thing about the microscopical structure of the umbilical vein in the new born lamb is the variability in the amount and distribution of the longitudinal muscle fibers of the media. In its extra-abdominal or cordal portion the structure of the vein was often found practically identical with that of the distal extremities of the retracted arteries. The lumen of the contracted vessel may be more or less stellate, crescentic, or a mere slit not infrequently shaped like the printed capital I. There is a single-layered endothelium. An elastica interna was observed several times in sections stained with Van Gieson and with orcein and fuchsin. The media contains longitudinally disposed muscle fibers which are somewhat irregularly distributed, and between which elastic tissue is found. However, none

of the longitudinal fibers are found grouped in bundles, among the fibers of the much thicker circularis as is the case in the arteries as a rule. The fibers of the circularis which are arranged rather loosely especially at the periphery, lie in concentric layers, those in the peripheral layers being more definite. No true adventitia can be said to exist although a number of small blood vessels are occasionally found in the small amount of cordal tissue that still surrounds the extra-abdominal portion of the torn veins.

The specimens of the intra-abdominal contracted portions of the paired veins which were examined have a very similar structure except that almost no circularis was present and that the fibers are interlacing. But the unpaired portion contrasts strongly with the former and the extra-abdominal portion. The unpaired portion is usually only partly collapsed and not contracted. Its walls are folded slightly and differ from the more distal portions in the entire absence of the longitudinal musculature, and in the occurrence of great variations in the thickness of the circularis. Although composed of much more closely apposed fibers the uncontracted circularis seems unusually thin for the size of the vessel and is surrounded by a fairly definite and vascular adventitia. In describing the umbilical vein in the human fetus Herzog stated that the wall of the vein is formed only by a well-developed media composed of fibrous connective tissue in which non-striated muscle is distributed very irregularly. Such an extremely irregular arrangement of the muscle fibers was seen in the umbilical vein of a dog but two days old and also in the hypogastric arteries of a newborn dog. In the first specimen the muscle was so loosely and irregularly disposed that it looked not unlike loose fibrous connective tissue and the adventitia which contained a number of small para-umbilical veins was thin and absent altogether in some places. Interlacing muscular fibers were also found in both the paired and unpaired portions of the vein of one lamb, however, and this interlacing is not infrequently so extensive that no regularity in the arrangement of the fibers of the media into circularis and longitudinalis can be distinguished. The non-striated

muscle fibers spoken of by Herzog are said to be disposed longitudinally or transversely, those extending in both directions lying adjacent and interlacing with each other. Herzog found no *elastica interna* and no elastic tissue present in man and Hauptmann reported similar results for the vein in the cord of the colt. The last author also stated that a strongly developed adventitia was present in the developing artery but at no stage in the development of the vein which was said to be in loosest connection with the surrounding tissues.

It is interesting that Haberda stated that all authorities are agreed that elastic tissue is sparse in the extra-abdominal portion of the hypogastric arteries and that even in their intra-abdominal portions less is found than in other arteries. Lochmann, on the contrary, who also worked with human material found an *elastica interna* present in all vessels and reported the formation of a new media in one case. However, Henneberg insists that Lochman's methods were not wholly reliable.

Although no special methods for demonstrating the presence of elastic tissue were used in every instance the presence of numerous elastic fibers in the distal extremities of several of the retracted hypogastric arteries of the sheep was unquestioned in specimens stained with Van Gieson even. They were not noticed regularly in sections of vessels from other animals stained in this way, however, and nothing that suggested the presence of a complete *elastica interna* was noticed save in some sections. Although occasionally very evident, it was seldom co-extensive with the perimeter of the vessel but was noticeable only in small areas. Moreover, since the *elastica interna* was usually perfectly evident in sections stained with Van Gieson, in very much smaller arteries that lay in the neighborhood, it is to be doubted whether it is invariably present in the peripheral portions of the hypogastric arteries even. Although allowance must be made for the effect of degenerative changes this supposition is further confirmed by the use of special stains such as Unna's orcein and fuchsin and Weigert's elastic tissue stain. In a specimen from a lamb $5\frac{1}{2}$ weeks old so stained a suprisingly well-developed and extremely folded *elastica interna* which was

double in some places lay in the media quite close to the lumen of the hypogastric artery but separated from the latter by a distinct although thin layer of fibrous connective tissue. As shown in figure 17 (*b*) this elastic membrane was folded so strongly in some places as to form a large accumulation of loops or coils somewhat similar to, though more pronounced than, those pictured by Henneberg in the extra-umbilical portion of the vein of a human fetus. In addition to this very evident elastic membrane numerous very long, tortuous and thick elastic fibers (*c*) were also distributed throughout the media in far larger numbers than found by Henneberg in the human hypogastric vessels.

Aside from possible specific differences the more pronounced folding of the elastica interna and the greater tortuousness and thickness of the elastic elements in these specimens as compared with those found by Henneberg in man may, of course, be due to the fact that the latter used specimens from comparatively younger individuals. The extremely folded condition of the elastica in these atrophic vessels and in contracted vessels as well, seems to suggest that the range of contractility and of elasticity of the intima is a rather limited one. Hence when this limit is exceeded by the contracting and atrophying musculature of the media it is folded and re-folded passively. Since the elastica is apparently well-preserved after the musculature and intima have already begun to degenerate it can usually be stained without difficulty by special methods although the failure to detect it in all routine Van Gieson stains might seem to indicate that it disintegrates earlier than the media. It is particularly evident in sections stained with an aqueous solution of fuchsin and mounted in glycerine or by the orcein-fuchsin method of Unna, both of which methods also reveal numerous tortuous elastic tissue fibers scattered throughout the media.

Most of the hypogastric arteries examined had a structure in marked contrast to that of the unpaired portions of the umbilical vein. However, their structure is identical with that of the unpaired vein in so far as no elastica interna was usually noticed, in the presence of a single-layered endothelium and a

similar adventitia. But no part of the hypogastric arteries except the distal portions were found wholly without longitudinal muscle fibers. However, the longitudinal layer varied greatly in thickness and distribution. This variation in thickness Loehman ascribed to the differences in contraction of the vessel in man. Most of the longitudinal fibers lay internally near the endothelium but various-sized bundles were found scattered about irregularly in the innermost portions of the circularis and not infrequently good-sized bundles were also found in the outer layers. In addition to this peculiarity in distribution of the longitudinal fibers, a peculiar, coarse radial striation or better lamination as

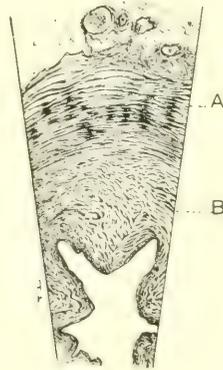


Fig. 24 Transverse section of the hypogastric artery of a new born lamb, showing the radial striation of the circularis. A, circularis, B, longitudinalis. $\times 97$.

shown in figure 24 was occasionally seen in the circularis of the arteries but never in that of the vein. This striation which was not present in all portions of the cross-sections was due to local accumulations of loosely-arranged circularly-disposed bundles of muscle fibers. Since these local accumulations lay approximately opposite each other they gave rise to the above mentioned coarse radial striations. Moreover, the fibers of the circularis were usually rather loosely disposed and the concentric strands separated from each other by a fine collagenous fibrous tissue.

The lumina of the arteries as in case of the paired portions of the umbilical veins, were roughly stellate. This characteristic

of the lumen was produced by a projection into it of thick welts or ridges or discontinuous longitudinal folds which however did not concern only the longitudinal fibers of the media although the contraction of these fibers, would, of course, result in an increase in their caliber in a direction at right angles to the lumen and hence would form folds which would have to encroach upon it or produce a dilatation of the vessel. The latter was, however, prevented by the active contraction of the circularis which tended to reduce the size of the lumen or even to obliterate it entirely and hence compress the longitudinal fibers. Since the latter need more room folding of the wall would hence seem to be the natural and inevitable result. It is interesting that Strawinski, von Hoffman and Herzog have described similar ridges in the unretracted hypogastric arteries in man. Hauptmann also found similar ridges in the noncontracted artery of the horse and states that they "may be as prominent as those described by Strawinski in man." Attention must be called to the fact, however, that there are apparently two kinds of ridges for Hauptmann attributed those found in the horse to large irregularly arranged, branched intimal cells 200 to 300 μ in size, which are surrounded by dense fibrous tissue and lie directly under the endothelium. Bucura, on the contrary, concluded that the longitudinal ridges are due to contraction of the longitudinal muscle fibers, while Pöllot asserted that the ridges and folds which project into the lumen of the ductus arteriosus are undoubtedly not produced by a contraction but are an expression of the unequal development of the constituents of the walls of the vessels. Moreover, Pöllot supports this opinion by results obtained from experiments on the aorta of guinea pigs. These consisted of the treatment of excised portions of the aorta with adrenalin, a procedure which failed to produce the ridges. But since the retracted and contracted hypogastric arteries and the umbilical veins in whose walls few longitudinal fibers were present also contain welts or ridges, and moreover, since they are absent in the non-contracted vessels or portions containing a large clot it is evident that they can be due to a contraction

even if not necessarily to a contraction of the longitudinal musculature alone as Henneberg concluded. Since the walls of contracted vessels as ordinarily seen in histological preparations are sinuous or folded more or less I cannot believe that what was seen in the retracted and contracted umbilical vessels is anything but an exaggeration of the same phenomenon and that both kinds of fibers when present are factors in their production for the circular fibers are very evidently even if not primarily, concerned. Moreover these ridges can be seen in degenerating umbilical vessels which are entirely free in their distal portions and the varying shape and size of these welts makes it impossible to attribute them solely to a varying development of the longitudinalis. Ridges or *Wülste* due to proliferation of the intima were never observed although their occurrence in the ductus arteriosus is not therefore denied.

A peculiarity often seen in cats two to four months old or older, and more rarely in young dogs, is the presence of one or more extremely fine long peritoneal bands about half a millimeter thick, extending from one portion of the mesentery to another and surrounding a number of coils of the small intestine. Taking their caliber into consideration these bands are unusually strong although fastened only at their ends and are apparently always bloodless. Since it is only occasionally that one end of these filaments is fastened to the peritoneum near the umbilicus it did not seem very probable at first that they were remnants of the omphalomesenteric vessels but since such delicate filaments were never observed in very young or old cats such an origin seemed not at all an improbable one. Hence two such strands from a cat ten weeks old, were examined microscopically but neither filament contained anything which could be recognized as a remnant of the omphalomesenteric vessels. Both were composed of fibrous connective tissue (fig. 25) containing one or two veins and several smaller arteries. By comparison it will be noticed that the structure of these cords is very similar to that of the temporary round ligament of some cats, rabbits and guinea-pigs. However, since these fine filaments

are occasionally eight to fifteen centimeters long in adult animals, such an origin would seem possible only on the supposition that considerable growth or elongation takes place in the vessels or filaments. Nevertheless, since the latter were observed in various stages of transformation such an origin becomes a highly probable one. This supposition was fully confirmed by a case in which one of the omphalomesenteric vessels was found attached to the apex of the bladder between the extremities of the hypogastric arteries in a cat about one year old. The end attached to the bladder had undergone fibrous transformation but the proximal portion still contained a remnant of the original lumen and



Fig. 25 Obliterated omphalomesenteric vessel from a kitten 18 to 26 days old. No remnant of the original lumen is visible and the whole structure is composed entirely of a vascular connective tissue. $\times 275$.

communicated with the superior mesenteric vein near the central end of the latter. In a second case, that of a cat one and a half to two years old a long, fine vessel containing blood throughout its entire extent was found with similar attachments. This vessel which ran among the coils of intestine likewise joined the superior mesenteric vein near its central end. It was quite uniform in diameter, about three-fourths of a millimeter thick, and took its origin in three fine veins on the sides and ventral surface of the apex of the bladder. Just cranial to the latter this surviving omphalomesenteric vein which had secured a secondary attachment was attached to the abdominal wall by an isolated fold of peritoneum 2.5 cm. wide and 2 cm. long although it was wholly free throughout the rest of its course. After

the death of the animal as the vessel cooled, the blood was slowly forced out into the superior mesenteric vein and the vessel then appeared only as a fine whitish cord. From these findings and from the similar behavior of the degenerating umbilical vein in dogs and sheep in which it not infrequently obtains a secondary attachment to the prominent fold of fat between the xiphoid and umbilicus, it is not only evident that the fine fibrous filaments frequently found in cats have such an origin. Moreover, it is evident that their persistence after birth is largely determined by the fact and more especially by the fact that they not infrequently come into relation with the systemic veins. It is also possible that the constant traction exerted upon them as a result of peristalsis in the contained coils of intestines is of some significance in this connection. Whenever they obtain connection with the peripheral veins the original lumen is preserved for a considerably longer time and may be wholly intact even after the musculature has become clearly degenerated. It is also evident that the arteries must undoubtedly degenerate first or earlier at least, for it is inconceivable that they could form part of the systemic circulation even if they obtained a peripheral attachment. That this is true is shown abundantly by the fact that in every case the persisting vessels containing blood were veins and not arteries. Moreover, no one has described a similar relationship of the hypogastric arteries which because of their large size and much later disappearance might be assumed to establish such a relationship far easier than the much smaller and far more functionally transient omphalomesenteric arteries.

Strangely enough such fibrous strands were never noticed in rabbits and no remnant of the omphalomesenteric vessels could be found in young rabbits more than 12 days old, while in guinea-pigs they are usually present at the age of three months or more. In the few newborn dogs examined not more than two strands of omphalomesenteric vessels were found and these were often twisted about each other so as to form a single strand which could be separated, however, as far as the lymph nodes at the root of the mesentery. If the two strands were distinct one of

them always ran farther caudally and it was this one in which blood could usually be seen. The other strand was shorter, firmer, whiter and looked more like an artery. Upon microscopical examination it was found that the single strand usually contained two vessels one of which might have the characteristics of a vein and the other that of an artery or they might be indistinguishable. In case of the second isolated strand which extends farther caudally the only vessel contained in it looked like a vein. Injection of these from the superior mesenteric vein, in cats one week old was easily accomplished.

Robin called attention to the presence of blood in the omphalomesenteric vessels in cats forty-eight hours after birth, described the course of the vessels and added that in some cats there might be three instead of only two vascular strands each containing two veins and two arteries. According to Robin the omphalomesenteric vessels of the cat detach themselves at the end of one month but as stated above, it is beyond question that not infrequently these degenerating vessels can be found attached to the umbilicus as late as six months and one year or more after birth.

Upon microscopical examination these undetached vessels were usually found to have fairly well-preserved walls with a distinct lumen. No *elastica interna* was observed and the musculature which had undergone regression was composed of indistinct circularly disposed fibers only. A definite adventitia was not present for it merged into the surrounding thick layer of fibrous connective tissue which was enclosed by peritoneum (fig. 26). The lumen was large, circular or elongated in cross section and contained some well-preserved erythrocytes as a rule. Each strand contained but a single large vessel and two vessels in each strand as reported by Robin were never seen except in regions where the two separate strands had joined. In case of the presence of three strands three vessels would, of course, be present here.

The apparent failure of the omphalomesenteric vessels to be completely transformed into fibrous cords before their complete

disappearance after becoming detached is probably due to the presence of degeneration and absorption similar to that which occurs in the umbilical vein in the dog and sheep. It is also interesting that no proliferation of the endothelium was noticed and that the musculature was comparatively well-preserved so long after the vessels were of any functional value to the organism. In some cases (fig. 12) the endothelium had been cast off, however, and plugged the small lumen. The cells which had undergone degenerative changes were not infrequently quite clearly outlined, somewhat stellate and formed a meshwork by the union of

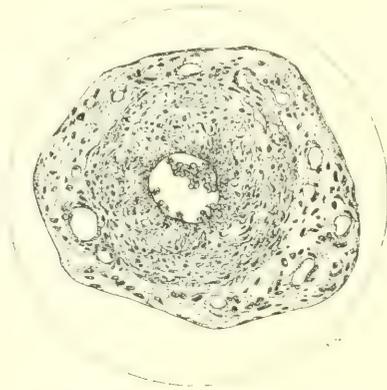


Fig. 26 Omphalomesenteric vessel from a pup 91 hours old. The arrangement of the inner portion of the wall suggests that of the original vessel. The lumen contains well-preserved erythrocytes. The outer lighter portion is composed of very loose fibrous connective tissue. The endothelium is intact in this portion. $\times 275$.

their processes. In other cases the lumen which contained well-preserved erythrocytes, and the bounding endothelium was well-preserved but the musculature had become so degenerated that it was difficult to recognize it as such. It had become syncytial and had lost its distinctive staining qualities besides being markedly vascularized at the periphery where it was partly or wholly replaced by connective tissue. Similar degenerative changes were also observed in the intima.

SUMMARY AND CONCLUSIONS

1. The umbilical arteries of ruminants very likely rupture extra-abdominally but at points which were intra-abdominal previous to traction.

2. Complete and immediate contraction of the arteries and of the extra-abdominal portions of the veins is made possible by the semi-fluid consistency of Wharton's jelly in these animals.

3. The suspensory and round ligaments of the liver are purely fetal structures in both the dog and the sheep. They degenerate very early in life and a true round ligament never exists in them.

4. Portions of both of these structures persist somewhat longer in cats rabbits, and especially in guinea-pigs and rats in which a more or less permanent round ligament is formed but neither ligament was ever seen in old cats and rabbits.

5. Degeneration and disappearance of the umbilical vein proceed centripetally and remnants of the original lumen are not necessarily preserved in the more or less temporary round ligament which may be formed in some of these animals.

6. The omphalomesenteric veins persist for an unusually long time after birth, especially in cats and they and the degenerating umbilical vein—except in the dog and sheep—may come into relation to the peripheral venous system. In case of the omphalomesenteric veins the establishment of this relationship is always preceded by detachment at the umbilicus and secondary attachment elsewhere. The detached degenerating umbilical vein of the dog and sheep may also obtain such an attachment but it never comes into similar relationship with the peripheral venous system.

7. No thickening or proliferation of the endothelium of the intima was observed in the umbilical and omphalomesenteric veins or in the hypogastric arteries of any of these animals and thrombosis was not a factor in the process of obliteration.

8. Fibrous transformation of the hypogastric arteries is due to degeneration of the media accompanied or followed by an ingrowth of connective tissue which is directly continuous with the pre-existing, sub-intimal, intra-medial or adventitial connective tissue.

9. The presence of an *elastica interna* is subject to some variation but it is usually easily demonstrable even in the extra-abdominal portions of the arteries and veins of the sheep in which the media contains many elastic fibers.

10. Embryonic regression of the media of the umbilical vein occasionally begins before birth and a budding or streaming of the syneytial-like media into the lumen was also observed.

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¹ Since this article was completed by September 26, 1913, the last year's literature is not referred to.

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