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THE STRUCTURE OF THE MAMMALIAN ŒSOPHAGUS.

BY

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WITH 17 FIGURES.

One of the most interesting features in the structure of the Mammalian Œsophagus is the extreme variability in the degree of development of the œsophageal glands in different species. For example, according to Ranvier (84) and others, the œsophagus of the rabbit, guinea-pig and rat is wholly devoid of glands, while in the dog, a thick layer of mucous glands nearly filling the submucous coat is found throughout the whole extent of the organ. Nor is this disparity confined to species belonging to different orders, for the cat and dog among carnivora and the sheep and pig among ungulates present equally striking differences in this respect.

The reasons which have been advanced for this disparity are based on the assumption that the function of the œsophageal glands is to furnish a secretion which will serve to lubricate the surface of the œsophageal mucous membrane and so facilitate the passage of the bolus of food in deglutition. Assuming that the secretion of the œsophageal glands possesses this purely mechanical function, the logical conclusion is that their development will be influenced by several factors which determine the consistence of the food-bolus which is to be swallowed, such for example as the character and bulk of the food itself, the efficiency of the masticatory mechanism, and the relative development of the salivary glands furnishing a secretion by means of which the food is diluted and rendered of softer consistence. Accordingly, various attempts have been made to establish a correlation between the degree of development of the salivary glands and the efficiency of the masticatory mechanism on

the one hand, and the number and size of the œsophageal glands on the other hand. For example, Ranvier (84), speaking of the difference between the rodents and the dog in this respect, remarks that this is easily comprehensible when one remembers that in the rabbit and other rodents the bolus of food is liquid or semi-liquid, and therefore mucous glands are not necessary, that on the other hand the dog swallows greedily solid matters and untriturated bones and hence the glands are indispensable. Renaut (97) similarly calls attention to the difference in masticatory efficiency between the ox and rodents on the one hand and the dog on the other and explains on this basis the differences in the number of œsophageal glands.

Many examples can be found in favor of this explanation. When, however, one attempts to give it a general application, unexpected difficulties arise. For example, there is not a sufficient difference in the development of the salivary glands, of the masticatory mechanism, or in the consistence of the food, to explain adequately the fact that glands are very numerous in the œsophagus of the dog and wholly absent from that of the cat.

The possibility that the mechanical function of the œsophageal glands may be a purely subsidiary one, and that their true function may be something quite different from this, has received but little attention at the hands of the investigators. Rubeli (90), it is true, suggested that the secretion might be of use in digestion, but his suggestion was based not on experimental data derived from mammals but on the observations of Decker (87), Swieçicki (76), Langley (79), etc., on the formation of proteolytic enzymes in the œsophagus of fishes and batrachians. As will appear later, these observations deal with structures which are not homologous with the œsophageal glands of mammals and can therefore not be used to draw conclusions concerning the function of the mammalian œsophageal glands. In this connection a question of much importance which has been variously answered by different observers is whether the œsophageal glands of mammals are pure mucous glands, or mixed glands containing serous demilunes. If serous cells are present, then one must at once think of a chemical function of the œsophageal secretion as well as a mechanical one. Klein (79) asserts that

demilunes occur in the œsophageal glands of the dog. Renaut (97) describing the œsophageal glands of the dog and of man makes the statement that they may be seen in preparations stained with his glycerine-hæmatoxylin-eosin mixture, without specifying whether he found them in one or both species. Schaffer (97) denies their presence in the human œsophageal glands and Rubeli (90) failed to find them in a number of domestic mammals. Stöhr (87) appears to have seen demilunes in his preparations, but to have interpreted them in accord with his well-known phase-theory as inactive mucous cells. More recently Helm (67) has described typical demilunes in the dog and pig and has demonstrated in them, by means of the iron-hæmatoxylin method, the intercellular secretion canaliculi.

Assuming that the function of the secretion of these glands is in part at least that of mechanically aiding deglutition, one would expect that in those cases where the character of the food and the nature of mastication suggest the need of these structures, but where, notwithstanding, no œsophageal glands are present, a compensatory development of other structures will be found, as, for example, a thickening of the stratified epithelium or an increased development of the muscularis mucosæ or of the external muscular coat. In other words, it is to be expected that a correlation of some sort will be found between the relative development of these structures and that of the œsophageal glands.

Accordingly, it seemed desirable to extend the investigation of the structure of the œsophagus to a much larger series of animals than has been considered hitherto by any single investigator, and to determine, as accurately as may be, the specializations which have arisen as a result of the response to the differences in the nature of the food on which these animals subsist. Among these, it might reasonably be expected that animals which live on coarse vegetable food would develop either a thickened epithelium, or a more completely cornified epithelium, or a layer of glands furnishing a lubricant secretion. It is, however, not by any means easy to estimate the degree of cornification of an epithelium except in those cases where a true stratum corneum composed of cells which have lost their nuclei is present. The thickness of the epithelium too is variable

from animal to animal of the same species, and in a single animal varies with the degree of extension of the membrane upon which it rests. Only the more pronounced differences in these respects may therefore be interpreted with caution from the standpoint of specialization.

It was apparent from the outset that very little help in interpreting the œsophageal glands of mammals could be obtained from the consideration of these structures in lower vertebrates, because glands occur in reptiles only in the form of imperfect crypts in certain turtles, and the so-called œsophageal glands of certain batrachia are, according to Bensley (00), in reality gastric glands.

Methods of Study.

One difficulty that at once presents itself in studies on the œsophagus is that of determining the point of transition of the pharynx into the œsophagus. In animals like the dog, where there is a transverse fold of the mucous membrane, corresponding in its position to the lower border of the cricoid cartilage, and to an actual change in structure of the mucous membrane, this is relatively easy, but in the majority of cases no such superficial line of demarcation exists, and the point of transition must be more or less arbitrarily established. In the descriptions which follow, the lower border of the cricoid cartilage has been taken as the point where the pharynx passes into the œsophagus. In his recent article on the œsophageal glands Haane (05) places the point of transition somewhat higher, at the level of the corniculate cartilage, but designates the portion of the tube included between this point and the lower level of the cricoid cartilage in the dog as "Œsophagus-vorraum." In order to include the doubtful region so designated by Haane, sections from this region have been studied in each mammal examined, but structures occurring above the distal margin of the cricoid cartilage have been referred to the pharynx, those below to the œsophagus.

In the case of the smaller mammals the entire œsophagus was fixed, in the larger mammals, where this was out of the question, a strip was taken including the whole length of the œsophagus.

For fixation, Zenker's fluid was employed, Bensley's alcohol-

bichromate-sublimate mixture being used where a more detailed study of the glandular epithelium was desired. The entire œsophagus or a strip, after fixation, was cut into lengths of 1 cm. to 2 cm., and imbedded in paraffin. From each of these segments, which were numbered consecutively from above downwards, sections were made at intervals of one millimeter, so that all portions of the œsophagus were examined. This method, however, did not exclude the possibility in the case of those animals where the results as regards the presence of glands were negative, that some glands were missed in the short unsectioned portions. Accordingly the following method devised by Bensley was employed, where the material was available, to make preparations in toto of the layer containing the glands, staining the latter selectively so that every gland lobule in the œsophagus was demonstrated clearly. The œsophagus was pinned out on cork and placed in 70 per cent. alcohol for 24 hours. Then, after a further stay of 24 hours in 95 per cent. alcohol, the mucous membrane was dissected off, by dividing the tela submucosa carefully with a scalpel close to the muscular tunic. In this way all the glands come off with the layer of submucosa which remains attached to the mucous membrane. The mucous membrane is placed in water for one hour, then transferred to a mixture of one part of strong muchæmatein (see Bensley, 03) and five parts of distilled water. In this staining solution the membrane remains for 48 hours, after which it is washed in distilled water and transferred to 95 per cent. alcohol containing two volumes per cent. of strong hydrochloric acid. In this solution the preparation remains until the glands stand out distinctly blue on a red background, when the preparation is washed in several changes of alcohol, dehydrated in absolute alcohol and cleared in benzole. Where the epithelium is thick, as in man, dog, etc., it stains so intensely that it interferes seriously with the transmission of light through the preparation. It is easy, however, to remove the epithelium by stripping off with forceps after clearing in benzole. By this means a preparation is obtained in which every gland of the œsophagus is clearly visible and their general relations to one another, the nature, course, and branching, of their ducts may be seen. Furthermore, in such prep-

arations the branching of the tubule may be studied with ease, thus avoiding the laborious method of reconstruction from sections. Because of the lack of sufficient material I was unable to apply the method in the case of the wild animals whose œsophagi were examined, but such preparations were made of all the domestic animals and of man.

For staining sections hæmatoxylin and eosin, iron hæmatoxylin, copper chrome hæmatoxylin, neutral gentian, muchæmatein, mucicarmine, Mallory's connective tissue stain and acid violet-saffranin were employed.

Opossum *(Didelphys virginiana).*

The mucous membrane of the œsophagus of the opossum exhibits the usual transitory longitudinal folds observed in the empty œsophagus. About 1 cm. above the cardiac orifice of the stomach, however, these disappear, and their place is taken by permanent transverse folds of the mucous membrane approximately 0.5 mm. in width, and provided on their free surfaces with a network of secondary ridges. These folds are separated from one another by deep sulci and, as will appear later, owe their occurrence in part to the accumulation in the lamina propria mucosæ of masses of glands.

The epithelium of the œsophagus at its upper end is represented in Fig. 1. It consists of a layer of somewhat irregular thickness, owing to the projection into it, from below, of ridges longitudinal in direction, belonging to the lamina propria. In full grown animal weighing 3,000 grammes, the thickness of the epithelium at the level of the cricoid cartilage was, in the spaces between the connective-tissue ridges, 190-250 micra, on the summit of the ridges 72-110 micra. The irregularity in thickness presented by this epithelium in transverse sections is due to high ridges of the lamina propria, which are for the most part longitudinal in direction, but are connected with one another by lower transverse and oblique ridges, so that in sections parallel to the surface at the level of the ridges a connective tissue network is seen surrounding islets of epithelium, instead of the epithelial network seen at this level in sections of the epidermis. As described by Oppel (97) in Phalangista, Phascolarctus and Aepyprymnus, true papillæ are wanting in the opossum.

On the surface of the epithelium a well-defined stratum corneum is seen, of fairly uniform thickness, although it dips down somewhat in the intervals between the longitudinal ridges of the lamina propria. This corneous layer presents two distinct strata, which correspond in a general way in their appearance and staining reactions to the stratum lucidum and stratum corneum of the epidermis. The deeper layer, 35 micra in thickness, stains deeply in eosin, particularly at the deep and superficial margins, the intermediate por-

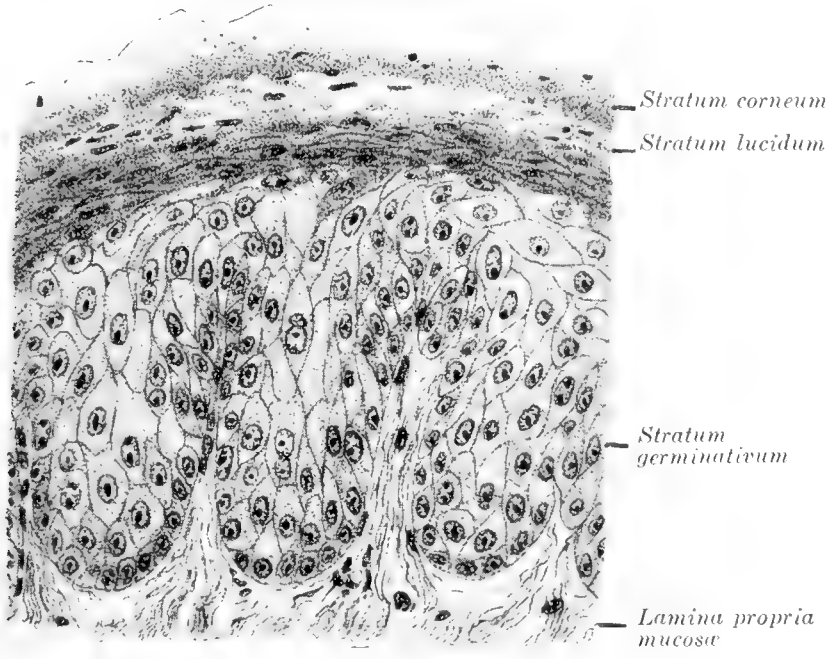

Fig. 1. Transverse section of epithelium of œsophagus of Didelphys at level of cricoid cartilage. × 120.

tion exhibiting, as is often the case with the stratum lucidum, patchy or irregular staining. This layer is composed of flattened cells, elongated, spindle-shaped in section, with a flattened nucleus rod-shaped in section. The superficial layer, 17 micra in thickness at its thickest portion, stains but faintly in eosin. It consists of cells of irregular polygonal shape similar to those of the stratum corneum of the epidermis with the exception that remains of the nucleus are to be found in them, in the form of shrunken remains of the nuclear membrane and one or two chromatic particles.

The stratum germinativum requires no special comment except that its superficial layers contain no granules of eleidin.

There is a gradual reduction in thickness of the epithelium going down the œsophagus. At the middle it is from 58-170 micra in thickness with a corneous layer 35 micra in thickness. The superficial layer of the corneous stratum here consists only of scattered cells of the sort described above.

At the point where the permanent transverse folds make their appearance about 1 cm. above the cardia there is a change in the character of the epithelium. On the surface and sides of these folds

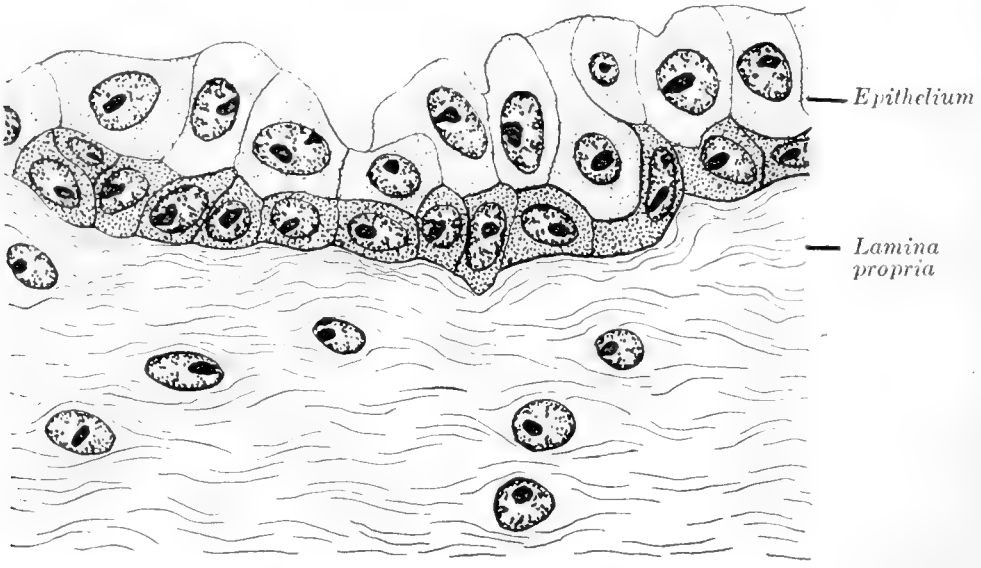

Fig. 2. Epithelium from side of transverse folds at lower end of œsophagus of Didelphys. × 750.

the epithelium varies from a double layer of cubical to polygonal cells, 10 micra in thickness (Fig. 2), to several layers of cells, the superficial layers flattened, 50 micra in thickness. The thin double layer of cells is found here and there on the sides of the transverse folds, the thicker epithelium on the summits. As shown in Fig. 2 there is frequently no sign at all of cornification of the superficial layer.

The lamina muscularis mucosæ is longitudinal in direction and is found throughout the whole œsophagus. At the lower level of the cricoid cartilage it makes its appearance as scattered bundles of

unstriated muscle. At the middle of the œsophagus it forms a continuous layer of considerable thickness (205 micra in an animal of 3 kg.) and increasing to 420 micra at the cardiac orifice of the stomach. In the lower portion, by reason of the fact that the glands are practically confined to the mucosa, the lamina muscularis mucosæ is separated by only a narrow band of collagenic connective tissue representing the tela submucosa, from the tunica muscularis.

Mucous glands are present throughout the whole length of the œsophagus and in the pharynx. In the upper part of the œsophagus

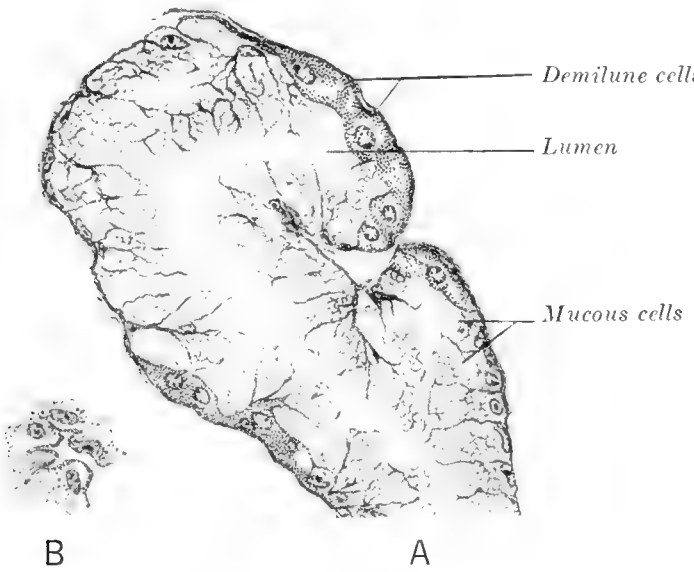

Fig. 3. A. Section of a tubule of an œsophageal gland of Didelphys showing mucous cells and demilunes. B. Group of demilune cells with central lumen and intercellular canaliculi. × 500.

they are located in the submucosa, but at the lower end where the transverse folds occur they are found in the lamina propria of these folds, superficial to the l. muscularis mucosæ, although here a few tubules may extend into the lamina muscularis mucosæ and even into the tunica muscularis.

The glands in all parts of the œsophagus of the opossum are mixed glands, that is to say they consist of mucous cells and demilunes or crescents. The latter are few in number at the upper end of the œsophagus, but at the lower end where the glands are located in the lamina propria mucosæ they are very abundant, as shown in Fig. 3.

The character of the mucous cells of these glands is well shown in Fig. 3 and requires no special description. Their secretory content stains selectively in muchæmatein.

The crescents (Figs. 3 and 4) are composed of cells which stain intensely in eosin. In the glands of the upper portion of the œsophagus they are to be found forming the characteristic crescent-shaped groups at the ends or along the sides of tubules. In the lower glands they form aggregates of considerable size, often surrounding a lateral diverticulum of the lumen of the gland so as to make a sort of sessile acinus on the side of a tubule. The character

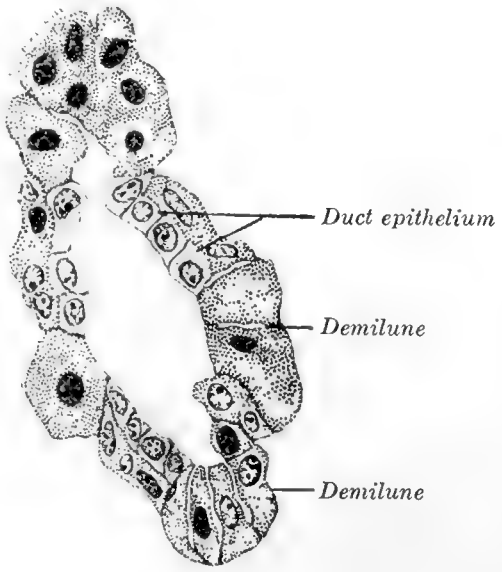

Fig. 4. Portion of duct of œsophageal gland from lower end of œsophagus of Didelphys showing demilune cells alternating with duct epithelium. × 500.

of the cells is the same at all points in the œsophagus. They are cuboidal in shape and the aggregate presents a rather remarkable resemblance to the parietal cells of the gastric glands. The cytoplasm is finely granular, but the granules are less crowded than in the parietal cells. Between the constituent cells of the complex, in iron hæmatoxylin preparations, may be seen fine intercellular secretion canaliculi, their outlines defined by fine cement lines. In Mallory preparations the granules along the canaliculi and on the lumen-border of the cell stain differently from the fine granules of the cytoplasm and probably represent the secretion antecedent of these

cells. There are no intracellular ductules. The crescents in the glands of the lower portion of the œsophagus are not confined to the mucous portion of the gland, but occur also in groups along the ducts, alternating with the non-secreting cubical epithelium of the ducts, as shown in Fig. 4.

The ducts of the glands are lined at their point of origin from the gland-tubule by a double layer of cuboidal cells. As they approach the surface the number of layers increases, there being a gradual transition to a stratified squamous epithelium. The majority of the ducts pass into the gland about the level of the deep border of the muscularis mucosæ. Frequently, however, they branch before penetrating the muscularis mucosæ, and occasionally they receive a small group of mucous tubules in the lamina propria or in the muscularis mucosæ. There are no aggregations of lymphoid tissue around the ducts nor are the latter enlarged to form ampullæ, as in the pig.

The ducts of the superficial glands at the lower end of the œsophagus are more numerous and the glandular masses are less complex. The ducts here open into the depressions formed by the secondary network of ridges on the surface of the folds and also at various points into the deep grooves between the principal folds.

The glandular tubules are supported by a thin basement membrane of reticulum, between which and the bases of the cell fine fibres resembling myofibrillæ may be seen. The latter stain red in Mallory's reticulum stain, and probably belong to stellate cells (Korbzellen), although this could not be demonstrated by the technique employed.

The tunica muscularis consists in the upper third of the œsophagus of striated muscle. At the middle of the œsophagus a few striated fibres are still to be found, but below this point it is all unstriated.

Rodentia.

The literature of this subject contains so many references to the structure of the œsophagus in Rodentia that it is unnecessary to describe in detail the conditions found in the different rodents examined. It will suffice to discuss those structures which may be con-

sidered to be specialized in some degree to meet the mechanical conditions imposed by the food.

The œsophagi of the following rodents were examined: Arctomys monax, Sciurus hudsonicus, Tamias striatus, Cavia, Erethizon dorsatus, Mus decumanus, Geomys bursarius, Lepus cuniculus, Lepus nuttalli mallurus. In the case of the porcupine (Erethizon) (Fig. 5 A) only the lower portion of the œsophagus about three centimeters

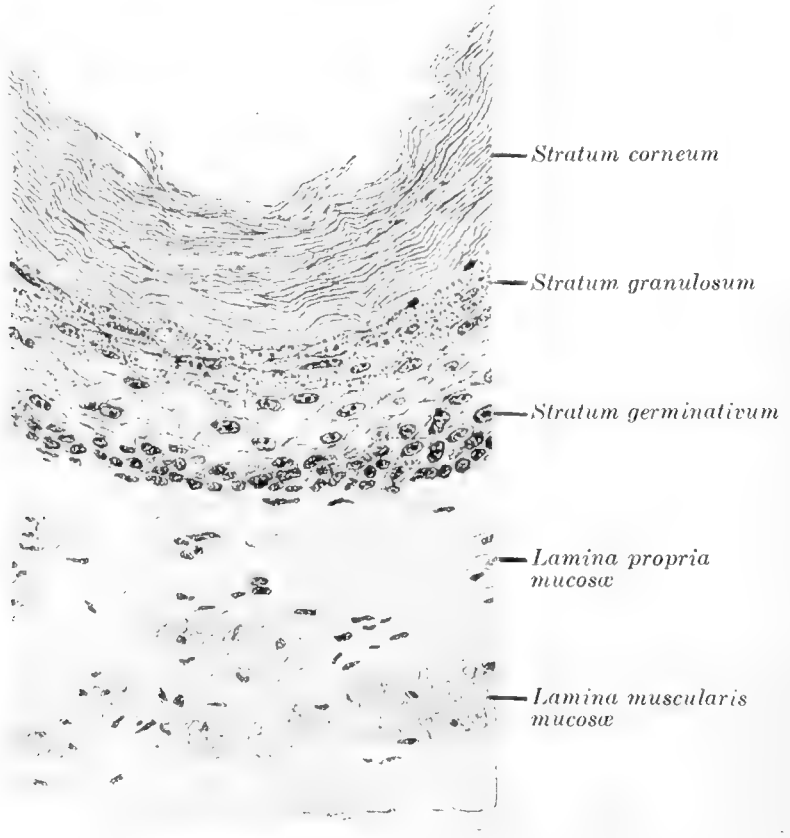

Fig. 5. A

Transverse section of tunica mucosa of œsophagus of Erethizon.

in length was available. In addition to the above genera the following rodents have been described by other authors: Mus musculus, Arvicola amphibius and Arvicola arvalis, Hypudæus arvalis, Spermophilus citillus.

With regard to the occurrence of glands, the results of all observations hitherto reported are negative except in the case of the rabbit, where glands are reported by Graff (80) and by Vogt and Yung (94), denied by Klein (71).

My observations are in accord with those of Ranvier (84), Brümmer (76) and others who deny the presence of œsophageal glands in rodents. In none of the species mentioned above are œsophageal glands to be found below the level of the cricoid cartilage. In the rabbit, pocket gopher (Geomys) and chipmunk (Tamias) groups of mucous glands were found above this layer in the submucosa of

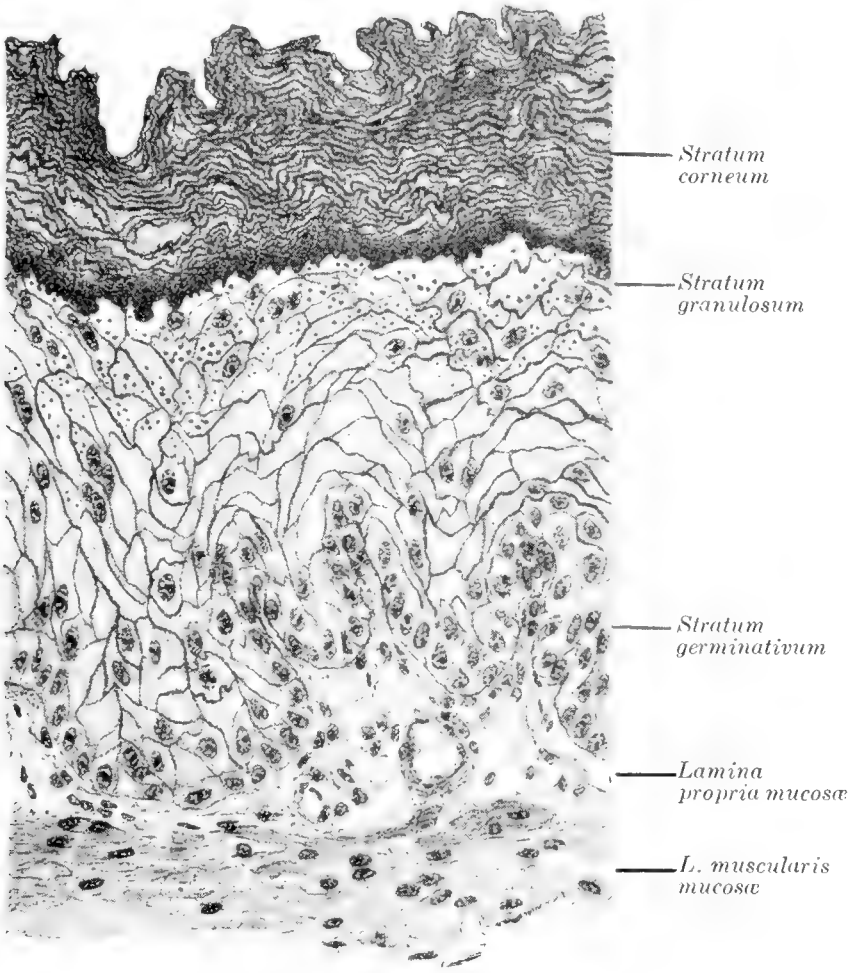

Fig. 5. B

Tunica mucosa of œsophagus of guinea pig. Showing true stratum corneum and stratum granulosum. × 120.

the pharynx, and it is probable that the occurrence of glands in this portion of the pharynx is responsible for the statements of Graff and of Vogt and of Yung that glands occur in the œsophagus of the rabbit.

The œsophageal epithelium in the rodents is a thick layer of stratified squamous cells presenting in the different members of the order two main types. One type (Fig. 5 A) is characterized by a very rapid

and complete cornification of the superficial layers of the epithelium to constitute a true stratum corneum composed of flattened cells wholly devoid of nuclei, and comprising over one-third of the total thickness of the epithelium. Below the stratum corneum in this type a true stratum granulosum is found consisting of two or more layers of cells, of elongated fusiform shape in section, containing numerous granules of eleidin staining blue in hæmalum. The stratum germinativum is relatively thin as compared with that of the second type. This type of epithelium is found in the guinea pig (Fig. 5 B), rat, pocket gopher and porcupine. The second type of epithelium (Fig. 6) also shows a considerable degree of cornification, but a true stratum corneum is not present, the superficial layers of flattened cells being nucleated and no stratum granulosum being found. In this epithelium the process of cornification appears to go on more gradually than in the first type and no sharp line can be drawn between the stratum corneum and the stratum germinativum. The superficial half of the epithelium nevertheless consists of flattened cells with elongated nuclei and stains more strongly in eosin than the deeper layer. Comparing this epithelium with that of the dog it is apparent that the degree of cornification is much greater in the rodent and that the thickness of the epithelium considering the relative sizes of the animals is much greater. True papillæ of the lamina propria mucosæ are not present in the rodents examined. In Cavia, Arctomys, Sciurus, Tamias and Lepus, however, as described by Strahl (89), the lamina propria mucosæ projects into the epithelium in the form of irregular ridges for the most part longitudinal in direction, and in Cavia and Tamias these have irregular summits which in places approximate the formation of papillæ. In Mus, Geomys, and Erethizon, there are neither ridges nor papillæ.

The l. muscularis mucosæ is present throughout the whole length of the œsophagus in all the rodents examined. It is particularly well developed in the squirrels, where it forms a continuous layer around the œsophagus.

The tunica muscularis in the rodents consists of striated fibres throughout the greater extent of the œsophagus. In Cavia, Geomys, Mus, Tamias, and Lepus the striated fibres are found to the cardiac

opening of the stomach. In Arctomys striated fibers are found right up to the cardia, but for a very short distance at the lower end of the œsophagus are mixed with smooth muscle. In Sciurus also there is a short distance at the lower end of the œsophagus in which smooth muscle is found. In Erethizon the outer layer of

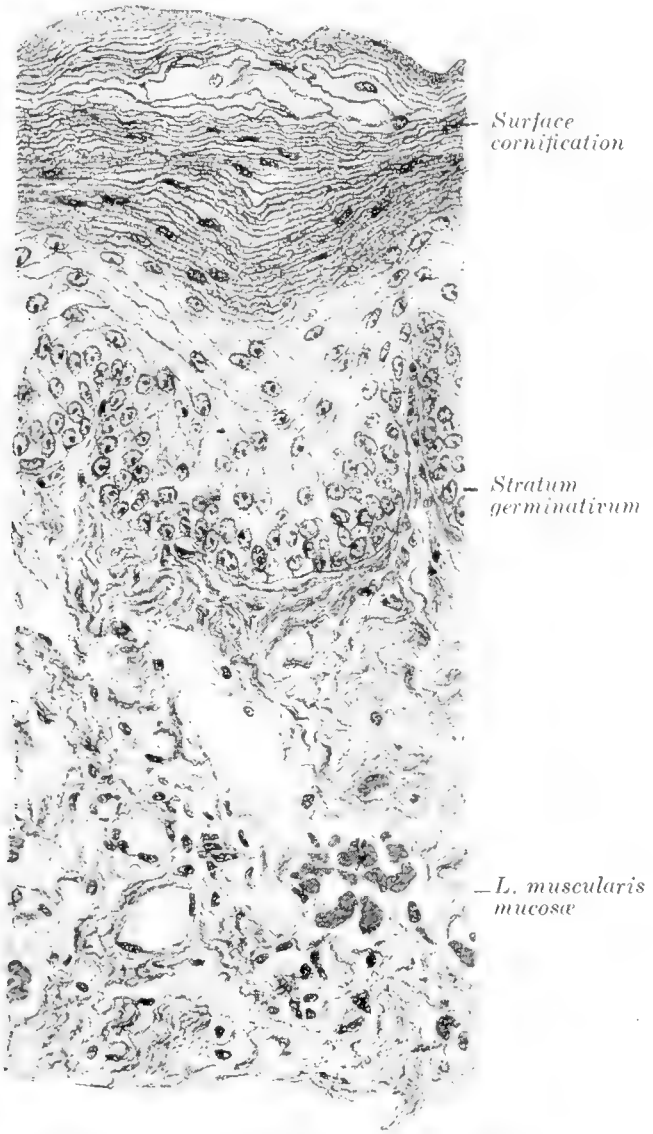

Fig. 6. Transverse section of tunica mucosa of Lepus nutalli mallurus. × 180.

muscle is striated to the cardia, but the inner layer has a thick lower sphincter composed of smooth fibres. In Muscardinus the striated fibres extend over onto the preventricular dilatation which, as is well known, contains fundus glands and is therefore to be regarded as a portion of the stomach.

Insectivora.

The only observations on the structure of the œsophagus in the Insectivora which I have been able to find are the descriptions of the œsophagus of Erinaceus given by Carlier (93) and Oppel (97). Carlier's description may be briefly summed up in the following statements: The epithelium is thick and of the ordinary type; the muscularis mucosæ is greatly developed consisting of large bundles of coarse non-striped fibers, longitudinally placed; the submucous coat is reduced to a minimum, due to the total absence of all glandular structures, mucous glands being entirely absent with the exception of a few scattered acini, situated near the cardiac end, internal to the muscularis mucosæ, and therefore in the mucous membrane. There are, however, according to Carlier, some serous glands in the submucosa of the organ, arranged in a ring round the cardiac orifice of the stomach, the long ducts of which pierce the muscle and epithelium to open just above the border of the œsophageal epithelium. The muscular coat consists throughout its whole extent of striped fibres. Oppel, on the other hand, found mucous glands in the upper portion of the œsophagus only. These glands showed cells of two types, as regards their affinity for hæmatoxylin and eosin respectively, although Oppel did not decide whether this difference was due to the physiological state of the cell or to a fundamental difference.

In Scalops aquaticus the conditions are very similar to those described by Oppel in Erinaceus. That is to say the glands are confined to the pharynx and to a very small portion of the upper extremity of the œsophagus.

The epithelium in Scalops is thick and fairly uniform in thickness throughout the length of the œsophagus, the increase in thickness towards the lower end of the œsophagus being but slight. At the upper end it measured 127 micra, at the lower end 139 micra. A true stratum corneum is not present, although a considerable degree of cornification of the superficial layers is apparent. The most superficial cells are nucleated and no stratum granulosum is to be seen. The superficial layers, about 24 micra in thickness, stain intensely in eosin. No true papillæ are present, although the deep

border of the epithelium presents a somewhat irregular outline in section.

The lamina muscularis mucosæ is well developed, except at the very beginning of the œsophagus. It forms a continuous layer composed of two or three layers of longitudinally disposed bundles of unstriated muscle.

The glands of the lower pharynx are mixed glands containing mucous portions with demilunes and serous alveoli.

The external muscular coat is composed of striated muscle throughout the whole length of the œsophagus.

Chiroptera.

This order is represented in my material by a single alcoholic specimen of Vespertilio fuscus, the brown bat.

The epithelium in the œsophagus of Vespertilio is thin (30-45 micra), and shows no stratum corneum, although the cells of the superficial two-thirds of the epithelium are much flattened. No papillæ are present.

The muscularis mucosæ is exceptionally well developed, reaching a thickness of 30 micra in the lower third of the œsophagus. It forms a continuous layer and consists of smooth muscle arranged longitudinally.

No glands are found at any level in the œsophagus.

The external muscular coat consists of striated muscle in the upper two-thirds and unstriated in the lower third.

Carnivora.

In the Carnivora the œsophagi of the following species, hitherto undescribed, have been examined: Procyon lotor, Lutreola vison, and Mephitis mephitica. One œsophagus of each species was available for this study including in the case of Procyon and Mephitis the whole of the œsophagus and the adjacent portions of the pharynx and stomach, in Lutreola the œsophagus and stomach only, so that in the latter animal the pharynx and transition region was not obtained. In addition the œsophagi of the cat and dog were examined to confirm the work of earlier observers. In these animals preparations

of the whole œsophagus were made according to the method outlined in the introduction in order to determine positively the distribution of glands in them. In the case of the cat three such preparations were made, all of which demonstrated the complete absence of glands below the level of the cricoid cartilage.

The œsophageal glands of the dog, like those of the opossum, are composed of two kinds of cells, mucous cells and serous demilunes. In this respect my observations confirm the statements of Klein (79), Renaut (97) and Helm (07) and are opposed to those of Rubeli (90)

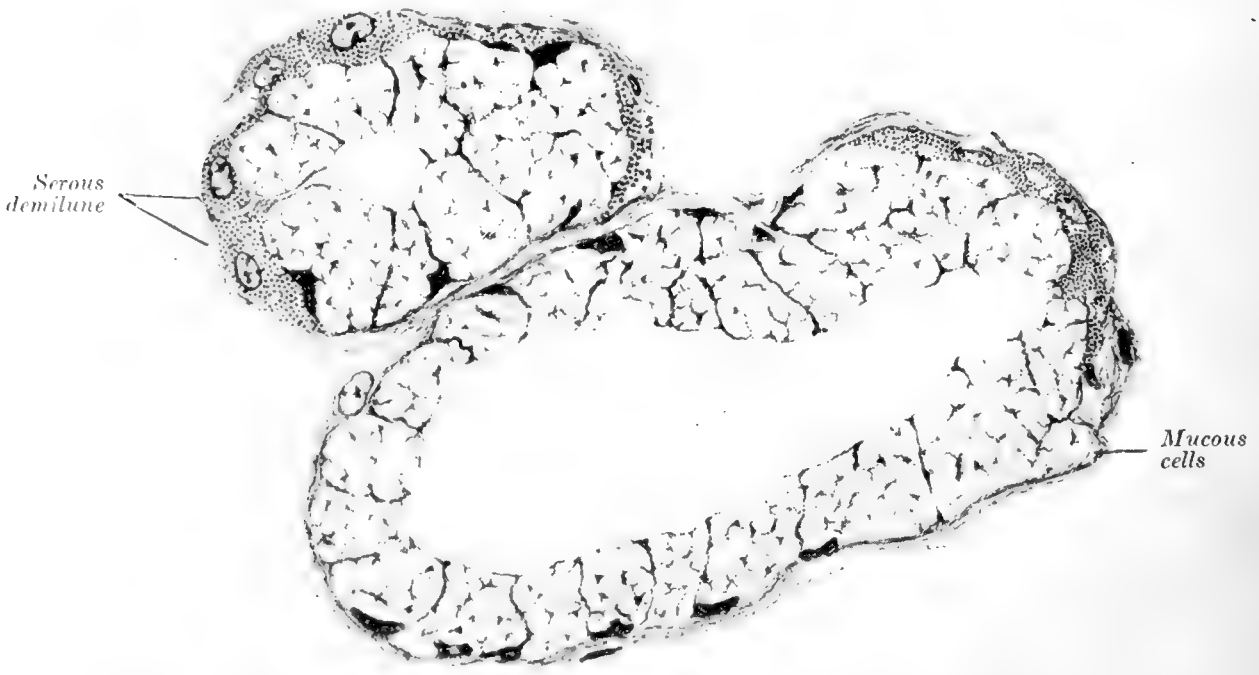

Fig. 7. Section of two tubules from œsophageal gland of the dog showing mucous cells and demilunes. × 750.

and Haane (05). The latter observer, it is true, does not specifically state that demilunes are absent, but implies it in the statement that he was unable to find intercellular secretion-capillaries. The demonstration of the demilunes in the œsophageal glands of the dog is no difficult matter if thin sections of well-fixed tissue are examined, and if an efficient differential staining method is employed, for they occur in relatively large numbers in all the œsophageal glands, although less numerous than in the glands of the opossum and raccoon (Procyon). For staining neutral saüreviolet-safranin is par-

ticularly valuable, the mucous cells staining red in this method, the demilunes violet. This method also shows well the intercellular secretion capillaries, although the iron hæmatoxylin method is preferable. The demilunes show the typical arangement as seen in the submaxillary gland and are provided, as indicated above, with

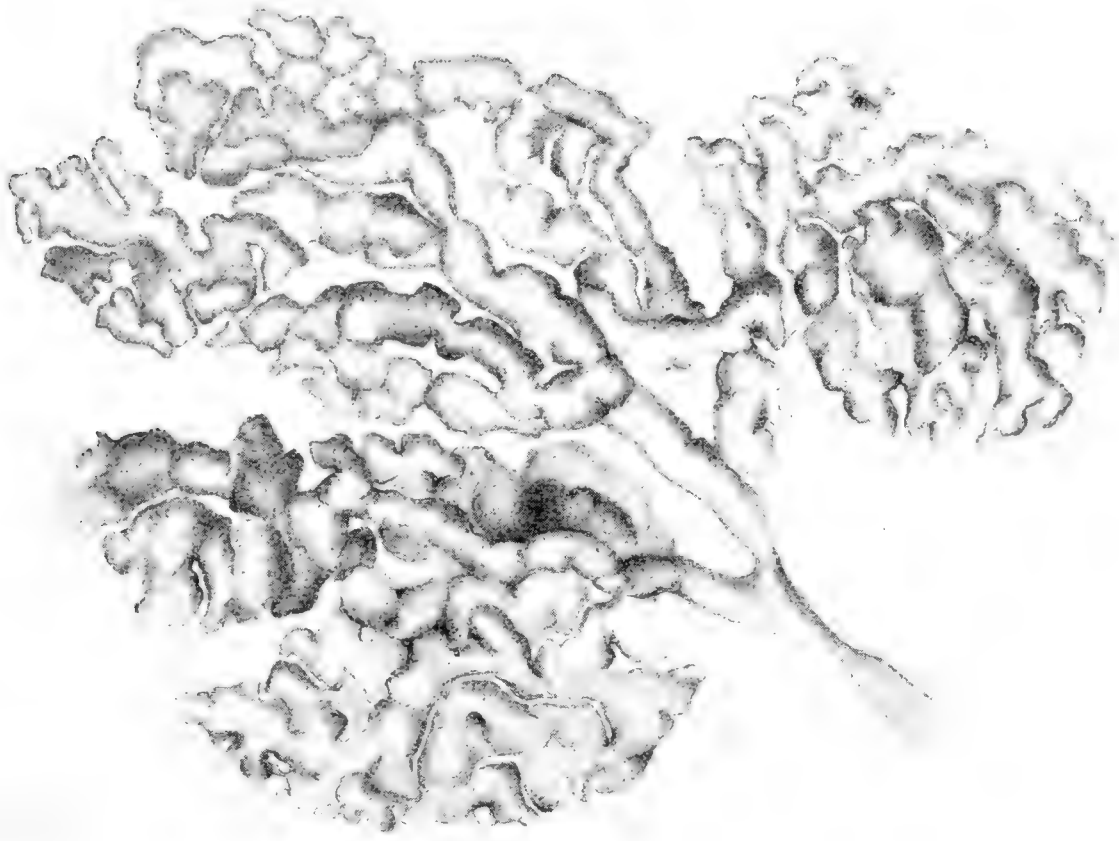

Fig. 8. Drawing of an entire gland from œsophagus of dog, showing tubulo-acinous character and mode of branching.

intercellular secretion capillaries resembling in every respect those found elsewhere (Fig. 7).

The shape and mode of branching of the gland is well shown in Fig. 8 drawn from a preparation stained in toto.

Procyon Lotor.

As in the dog, there is, in the raccoon, in the lower part of the pharynx, a circular fold of mucous membrane which projects into

the lumen and forms a superficial demarcation between the œsophagus and pharynx. This fold in the raccoon, however, is without glands.

The epithelium (Fig. 9) varies in thickness according to the degree of contraction of the surface upon which it rests from 158 micra to 220 micra. There is no marked thickening of the epithelium as the stomach is approached. The lower border of the epithelium is irregular in transverse sections owing to the projection into it of high ridges of the lamina propria mucosæ. These ridges are chiefly longitudinal in direction, but are connected with one another by numerous oblique ridges. On the summits of these ridges low conical papillæ are found.

The muscularis mucosæ is well developed throughout the whole œsophagus, consisting of two or more layers of longitudinal bundles of unstriated muscle.

Glands are present in the tela submucosa throughout the entire length of the œsophagus, and are fully as numerous as in the dog. The glandular lobules are ovoidal in shape, somewhat compressed from side to side and so placed that their long diameter coincides in direction with the long axis of the œsophagus. Many of the lobules have independent ducts, but in the majority of cases a duct divides below the lamina muscularis mucosæ into two ducts which enter adjacent lobules. The glands are of the tubulo-acinous type, the lobule being a system of highly branched tubules with small acini along their course and at their terminations. There is no difference between tubules and acini as to the character of the lining cells. The epithelium changes from duct epithelium to secreting epithelium at the point of entrance of the duct, so that all tubules within the lobule are lined exclusively by glandular epithelium.

The character of the glandular epithelium is well shown in Fig. 10. It consists of mucous cells and serous demilunes. The latter are very abundant and conspicuous. The mucous cells correspond in character with those of other mucous glands and require no special description. The demilunes in the glands of the upper part of the œsophagus have the characteristic crescentic shape in sections. In the glands of the lower portion of the œsophagus many demilunes

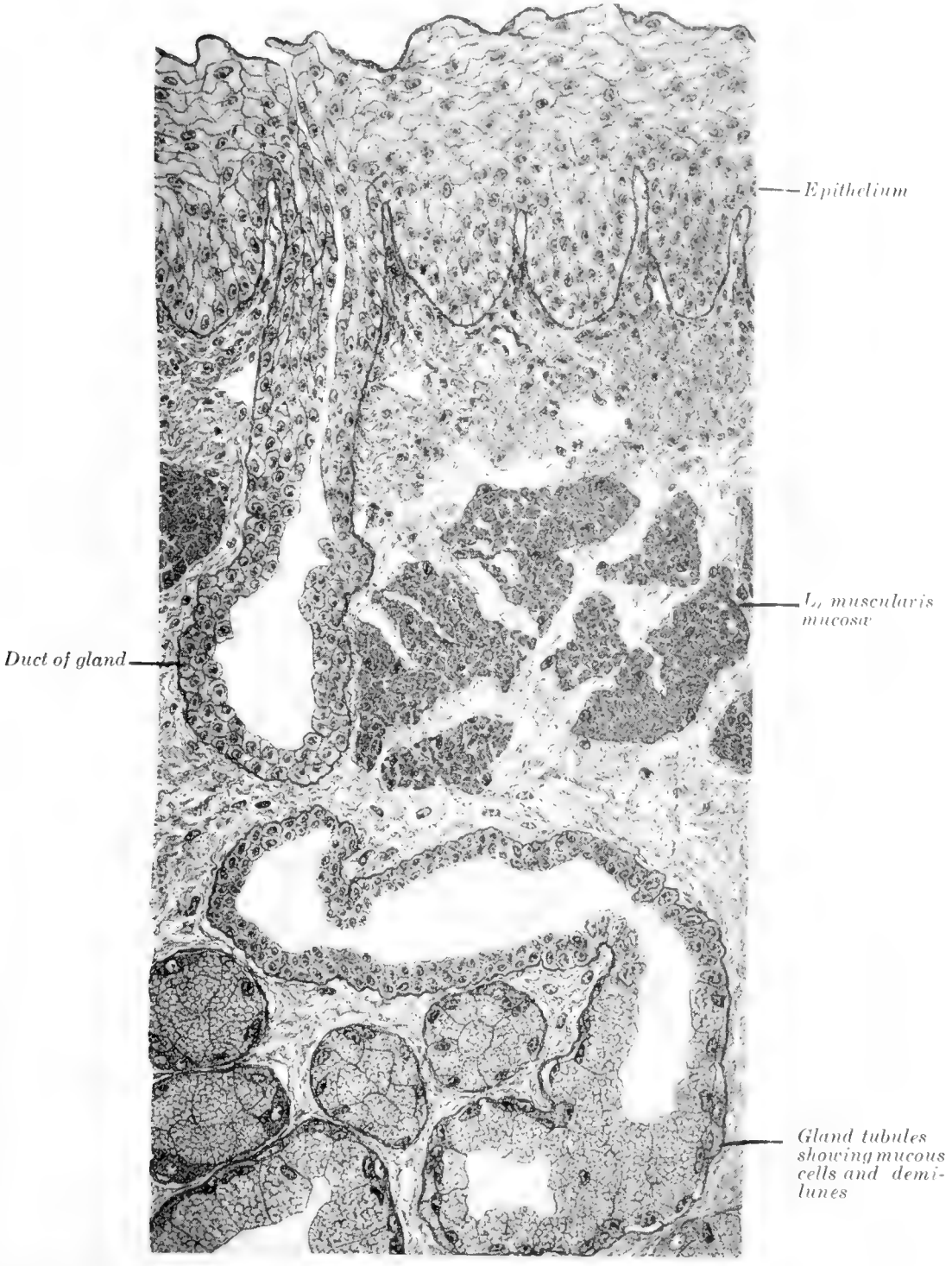

FIG. 9. Transverse section of tunica mucosa and part of tela submucosa of œsophagus of Procyon. × 120.

are so large that they form small sessile acini along the sides of the tubules. In both cases intercellular capillaries are seen in iron hæmatoxylin preparations, outlined by cement lines running between the adjacent surfaces of the cells. In the acinus-like demilunes of the glands at the lower end these intercellular ductules open into a central lumen which is directly continuous with the lumen of the main tubule. Although the material was not suitable for studying the secretory content of the cells, because of the fact that the œsophagus was fixed in Zenker's fluid fully an hour after the animal was

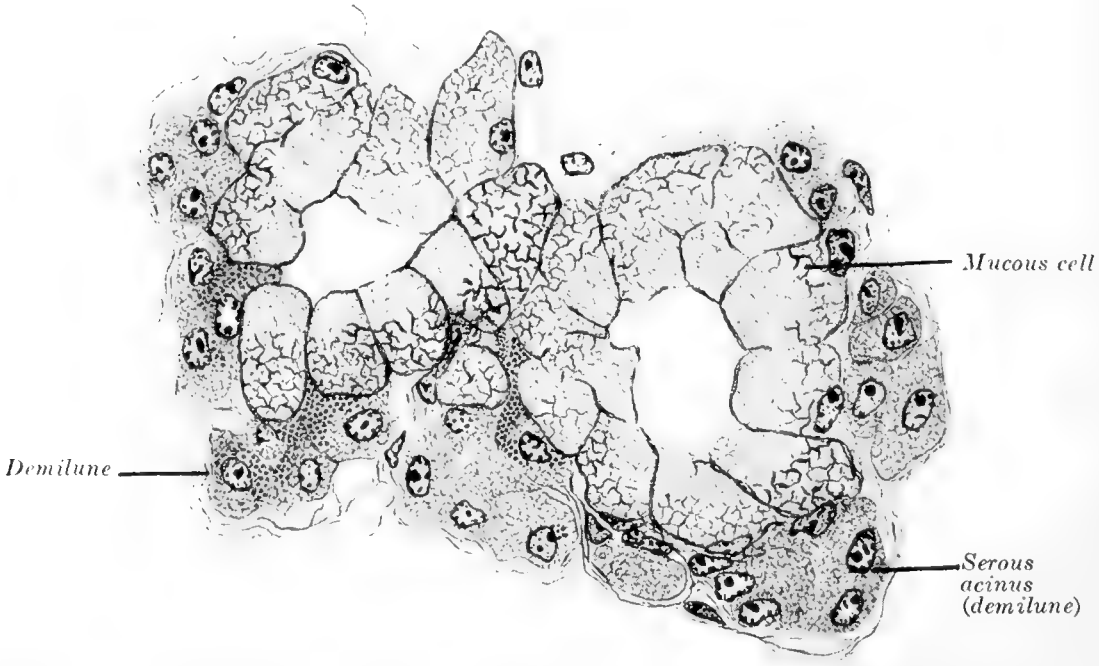

Fig. 10. Portion of tubule of œsophageal gland of Procyon showing mucous cells and large demilunes with secretion granules. × 500.

killed, yet in some of the more superficial glands the cells of the demilunes contained well-fixed secretion granules (Fig. 10), which stained strongly in eosin and in iron hæmatoxylin and which occupied the portion of the cells bordering on the lumen. No basal filaments, however, were demonstrated.

The ducts of the gland are lined for a very short distance at their origin from the lobule, by a low simple cubical epithelium. A short distance from its origin this changes to a two-layered epithelium, the cells of the surface layer having their long axes perpendicular to the basement membrane, those of the deep layer parallel to it.

In the upper part of the duct a third layer is added and the surface layer becomes flattened. The ducts enter the epithelium between the ridges of the lamina propria.

The ducts run perpendicular to the surface and have no ampulla-like enlargements. No lymphatic nodules nor accumulations of lymphocytes occur in relation to the ducts.

The tunica muscularis is composed of striated muscle throughout nearly its whole extent. In the stratum circulare smooth muscles make their appearance a short distance above the cardia and expand at the cardia into a lower sphincter.

Mephitis Mephitica.

In Mephitis the epithelium is extremely uneven in thickness owing to the fact that from the deep surface epithelial processes descend into the lamina propria mucosæ. The connective tissue separating these processes represents, somewhat more highly developed, the longitudinal and transverse ridges of the œsophagus of the raccoon. The thickness varies from 122 micra to 294 micra, the difference between these measurements indicating the height of the epithelial processes. The epithelium shows very little evidence of cornification, although the superficial layers of cells are much flattened and stain more readily in eosin than the deeper ones.

The lamina muscularis mucosæ begins at the upper end of the œsophagus as scattered bundles, but rapidly becomes a complete layer 47 to 118 micra in thickness, according to the degree of extension of the mucous membrane.

The only glands present in the œsophagus are found just at the cardiac orifice of the stomach. They are located in the submucosa and are similar in structure to the glands of the œsophagus of Procyon. The demilunes are particularly abundant and have the characteristic form and structure. The ducts begin in the lobules with a lining of cubical cells beneath which is an imperfect second layer of cells. This second layer quickly becomes complete on going up the duct and additional layers are added as the surface is approached, as in œsophageal glands elsewhere. These glands are therefore in

every respect typical œsophageal glands and can not be confused with the neighboring cardiac glands of the stomach.

The tunica muscularis consists of striated muscle throughout its whole extent.

Lutreola Vison.

The epithelium in Lutreola is very similar to that in the cat. No true papillæ are present and there is a very slight indication of ridges of the lamina propria so that the deep border of the epithelium is fairly regular. The cornification is imperfect but is indicated by the different staining of the superficial layers of cells which are much flattened, but which retain their nuclei. The thickness is fairly uniform throughout except for such differences as are the result of tension.

The lamina muscularis mucosæ is well developed, forming a layer 50-80 micra in thickness at the upper end of the œsophagus and gradually increasing in thickness to 180 micra at the cardiac orifice.

A few glands only are present, and these are confined to the upper fourth of the œsophagus. They are similar in nature to the glands of the œsophagus of Procyon, that is they consist of mucous cells and demilunes.

Ungulata.

My observations on the structure of the œsophagus of the ungulates have been largely confirmatory of the work of Ellenberger (84), Rubeli (90), Haane (05), and Helm (07).

To ascertain beyond doubt the presence or absence of glands in the œsophagus of the sheep and ox, preparations were made of the whole œsophagus according to the method already outlined, but with negative results. These œsophagi contained no glands. In the case of the horse sections made at different levels were likewise devoid of glands.

In the œsophageal glands of the pig, I have found, in confirmation of Helm (07), demilunes of serous cells, but in smaller numbers than in any of the other animals whose glands were studied with the exception of man. They are, however, by no means infrequent,

although the complexes are smaller in size, more compressed, and more easily overlooked than in other animals. These demilunes are provided with intercellular secretion-canaliculi.

In all the ungulates examined, as might be expected, the epithelium shows a marked thickening, and an unusual degree of cornification. The condition in the sheep as shown in Fig. 14 is typical of that found in the sheep, ox and horse. The epithelium is thick and presents on its deep surface an irregular outline owing to the presence of longitudinal ridges of the lamina propria mucosæ. In addition, both on the summits of these ridges and between them there are extraordinarily long papillæ, for the most part simple, which penetrate the epithelium as far as the stratum corneum and even penetrate that layer for a short distance. A thick stratum corneum forms the outer portion of the epithelium, forming from one-third to one-fourth of the entire thickness of the epithelium, the thicker portions being found in the lower regions of the œsophagus. In this stratum corneum two secondary strata may be made out which may be compared in general with the strata lucidum and corneum of the plantar skin, although there are important differences in structure. Both layers are very homogeneous and transparent in the fresh condition, and in unstained alcohol-fixed sections. The deeper layer is composed of fusiform flattened cells, but with flattened nuclei. The superficial layer shows the wrinkled cell borders seen in section of the stratum corneum of the skin. No true stratum granulosum is present, but the superficial portion of the stratum germinatum shows indications of a change preparatory to cornification, in that the cell protoplasm of this layer stains more readily in fuchsin than the deeper layers. Nuclei are present in the cells of the most superficial layers of the stratum corneum, although they are much degenerated. Thus while a deep stratum corneum is present the changes do not involve the nuclei of the cells to the extent that they do in the guinea pig.

In the pig, also, the epithelium is thick, but the degree of cornification is much less than in the sheep, the contrast between the stratum germinativum and the stratum corneum less striking, and the transition less abrupt from the one layer to the other. We do not see

in the œsophagus of the pig the division of the stratum corneum into two layers as in the sheep, and in the pig nuclei are more numerous in the superficial layers and less degenerated. There is thus in these animals a very evident relation between the presence of glands and the degree of cornification of the epithelium.

An interesting fact in connection with the structure of the pig's œsophagus is the arrangement of the muscularis mucosæ. In the upper portion of the œsophagus where the glands are abundant there is no muscularis mucosæ. It makes its appearance a short distance above the point at which the glands begin to thin out and is well developed in the lower half of the œsophagus where there are few glands.

Man.

Much disagreement exists between observers as to the number and distribution of the œsophageal glands in man. According to Toldt (89) they are present in large numbers in the upper section of the œsophagus and are wholly wanting in the lower segment. According to Klein (71) they are of rare occurrence. Kossowski (80) on the other hand finds them most abundant at the lower end of the œsophagus near the cardia. Dobrowolski (94) found that there were considerable individual variations, but that the whole number did not exceed two hundred, of which two-thirds were in the upper half of the œsophagus. The latter observer's results were based on preparations of the entire œsophagus and his conclusions as to the individual variability are supported by the preparations which I have made by the method already outlined. The extent of the individual variations is well indicated by figures 15 and 16 which show the exact location of every glandular lobule in two human œsophagi. One of these contained 741 lobules pretty well distributed over the whole œsophagus with the exception of a short area at the beginning of the œsophagus and another near the cardiac end where the glands are relatively few in number. The other œsophagus had but 62 glandular lobules, 58 of which were found in a segment 4 cm. in length beginning 3 cm. below the cricoid cartilage. In a third œsophagus there were 140 lobules of which 43 were located in a

segment 3 cm. long at the lower end of the œsophagus. Above this a segment 3 cm. long was free from glands. Above this 37 glands were distributed over an area 10 cm. in length. At the upper end of the œsophagus 60 glands were distributed over a distance of 7 cm., this group being separated from the middle group by a segment 2 cm. in length devoid of glands. In addition to the glands there were 54 cysts of various sizes. As the figure (15) indicates, there

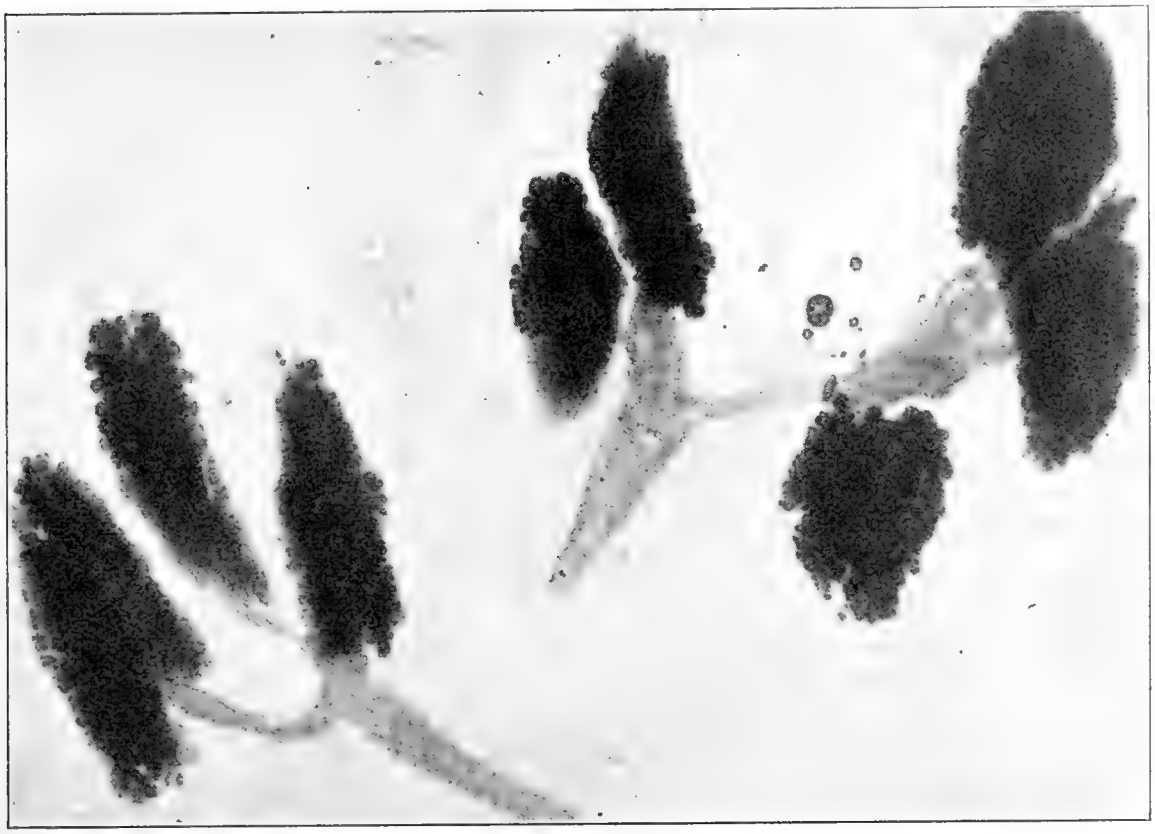

Fig. 11. Photomicrograph of two glands from human œsophagus stained in toto. The long axis of the œsophagus coincides with that of the glandular lobules and the ducts point obliquely toward the stomach.

is a marked tendency for the glands to be arranged in longitudinal rows parallel to the long axis of the œsophagus. These rows may be as many as eight in number at one transverse level and are not confined to the anterior surface and lateral surfaces as stated by Dobrowolski, but are distributed indifferently on all surfaces of the œsophagus.

The relation of the ducts to the lobules is well shown in Fig. 11.

Each duct is oblique in direction with reference to the axis of the œsophagus but has a general direction downward in the direction of the stomach. The descending portion of the duct lies in the tela submucosa close underneath the lamina muscularis mucosæ, and may reach a length of 4.6 mm., although the majority of them are much shorter. Each duct branches at its termination into from two to five rarely more secondary ducts, each of which enters a lobule. Ampullary dilatations of the ducts are of very common occurrence and affect most frequently the oblique portion of the main duct in the submucosa. Almost as frequent, however, are the dilatations into ampullæ of the secondary ducts at the point of emergence from the lobule or in the hilum of the lobule. The portion of the duct contained in the lamina propria mucosæ is frequently surrounded, as pointed out by Flesch (88) and Schaffer (97), by accumulations of leucocytes and often by true lymphatic nodules with a germinal center. At the point where the duct emerges from the lobule there is also, frequently, surrounding the duct, an accumulation of reticular tissue containing large numbers of leucocytes. The nature of the leucocytes found in these accumulations varies considerably, but in some cases the predominating cell is Unna's plasma cell. In other cases the lymphocytes predominate. In all cases, however, there are large numbers of plasma cells distributed along the duct and in the reticular tissue forming the framework of the gland, and in those cases where no nodule is present along the duct one finds occasionally the whole duct surrounded by a narrow layer of reticular tissue containing plasma cells and lymphocytes.

As regards the structure of the lobule and the character of the individual cells composing the gland, little can be added to the excellent description given of these structures by Schaffer (97) and the description which follows is largely confirmatory of his observations.

The lobular duct gives off within the hilus of the gland a series of intralobular ducts which are arranged radially around it and are of variable length, those which drain the portions of the lobule near the hilus being short, those which go to the terminal portions of the lobule longer. Each of these intralobular ducts drains a pyramidal

mass of tissue the apex of which is directed towards the duct, the base towards the periphery of the lobule. These pyramidal masses are the units of structure, being composed exclusively of tubules and acini lined by secreting cells. As indicated above, the gland is tubulo-acinous in type, the body of the unit composed of branching tubules which terminate in bulb-like acini, tubules and acini being occupied by the same type of epithelial cell. The acini show a slight expansion of the lumen and the cells are frequently longer in the acini than in the tubules, thus accounting for the difference in size.

To the question of the presence of demilunes in the œsophageal glands of man I have given much attention. In view of the fact that I have found these structures without difficulty in all œsophageal glands of other mammals it was my expectation that they would also be found in man. Accordingly I have studied carefully complete series of sections, 5 micra thick, of glands from three different œsophagi, using the methods of staining which I have found most efficient in demonstrating these structures in other mammals. The result of this study has, however, been wholly negative and I am forced to agree with Schaffer, who states that the human œsophageal glands are pure mucous glands without demilunes. Referring to the literature I find that neither Klein (79) nor Renaut (97), who are quoted in this connection by Schaffer and Oppel, specifically state that they found demilunes in the human œsophagus, although the former found them in the dog and Renaut's description applies in general to the dog and man. Böhm and v. Davidoff (95) state that there are but few mucous glands in the œsophagus but that when present they contain well marked demilunes, but it is difficult to determine whether these authors had before them the true œsophageal glands occurring in the submucosa, or the superficial glands of Rüdinger which have been shown by Schaffer (97) and Hewlett (00) to contain parietal cells and from which their illustration of the œsophageal glands of man is clearly taken.

The character of the mucous cells forming the secreting tubules is well shown in Fig. 12. Protoplasmic cells containing no mucin as described by Schaffer occur also in my preparations, whole groups

of tubules being of this character. These I interpret, as did Schaffer, as temporarily inactive mucous cells. These tubules are always surrounded by a tissue containing large numbers of plasma cells.

The ducts of the glands (Fig. 12) are lined by a stratified epithelium except in the very beginning in the gland where for a short distance, only 12 micra in some cases, a single layer of cylindrical cells is found. The intralobular ducts have a double layer of cells and the large ducts in the submucosa two to four layers of cells, the number of layers increasing as the surface of the mucosa is approached. The shape of the cells varies with the degree of tension of the surface, produced either by distension of the duct with secretion or by the

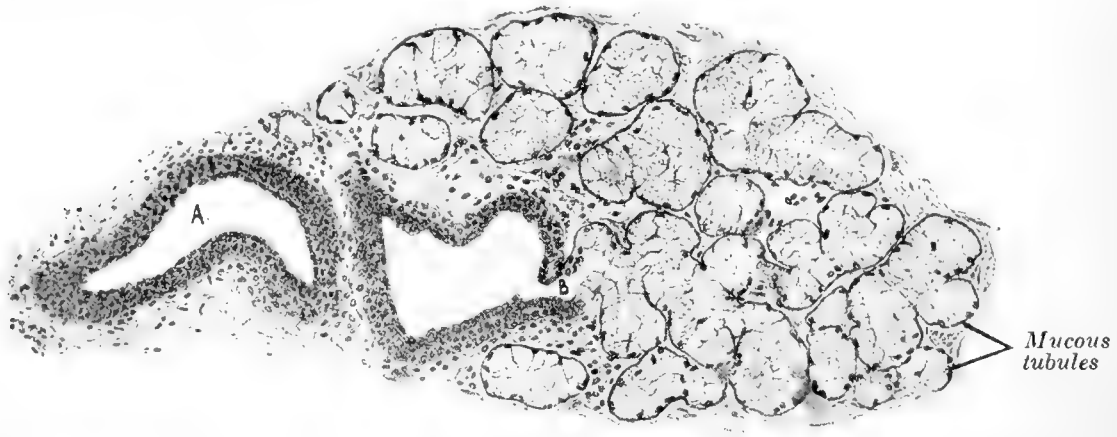

Fig. 12. Portion of a lobule of an œsophageal gland of man, showing entrance of duct and transition of duct epithelium to glandular epithelium. A. Lobular duct. B. Transitional duct lined by simple columnar epithelium. × 180.

formation of folds in the mucous membrane. In the relaxed duct the superficial layers of cells are cylindrical, the deeper layers polygonal except very near the epithelium, where the epithelium becomes stratified squamous. In the distended or stretched duct both superficial and deeper layers of cells become more or less fusiform in section. In all cases the superficial layers of cylindrical or flattened cells stain more strongly in eosin than the second layer, indicating a probable change in a slight degree of the protoplasm in the direction of cornification.

As regards the superior cardiac glands of Schaffer and Rüdinger I have been able to make no study of them owing to the absence of

suitably fixed material. In one out of four œsophagi of which total preparations were made to study the distribution of the glands, groups of these glands were found in the upper part of the œsophagus extending 4.5 cm. below the lower border of the cricoid cartilage. In this case twelve patches of these glands were found varying in size from 1 mm. to 15 mm. in length and from 1 mm. to 3 mm. in width.

The mucous tubules and acini are surrounded by a well marked basement membrane composed of reticulum, and the tubules are bound together by a reticular framework. No myoepithelial cells could be demonstrated between the basement membrane and the epithelium of the glandular tubules.

The epithelium in man is of considerable thickness, varying in this respect with age, and with the degree of tension of the mucous membrane. Another cause of variation in thickness in specimens of the human œsophagus is due to post-mortem loss of the superficial layers of cells owing to maceration. In the newborn child it has a thickness of 113 micra, in the adult from 260 to 440 micra.

The degree of cornification of the epithelium in man is about the same as in the pig and considerably less than in the sheep and ox, that is to to say that, although the superficial layers of cells show indications of chemical change in their greater transparency and in staining more readily in eosin, the degree of flattening of the cells is much less than in the animals mentioned, and oval nuclei, which stain well, are preserved even in the most superficial layers (Fig. 13).

The deep border of the epithelium is irregular owing to the presence of transitory folds of the lamina propria, described by Strahl (89) and more particularly owing to the presence of large numbers of high conical papillæ. The latter are arranged in linear rows running parallel to the axis of the œsophagus and resembling in their general arrangement those found in the skin of the palmar surfaces (Fig. 17). In some cases, as shown in the figure, the rows are composed of several ranks of papillæ, the individual papillæ being irregularly distributed.

The lamina muscularis mucosæ is well developed. It begins in the lower part of the pharynx as scattered bundles of longitudinally

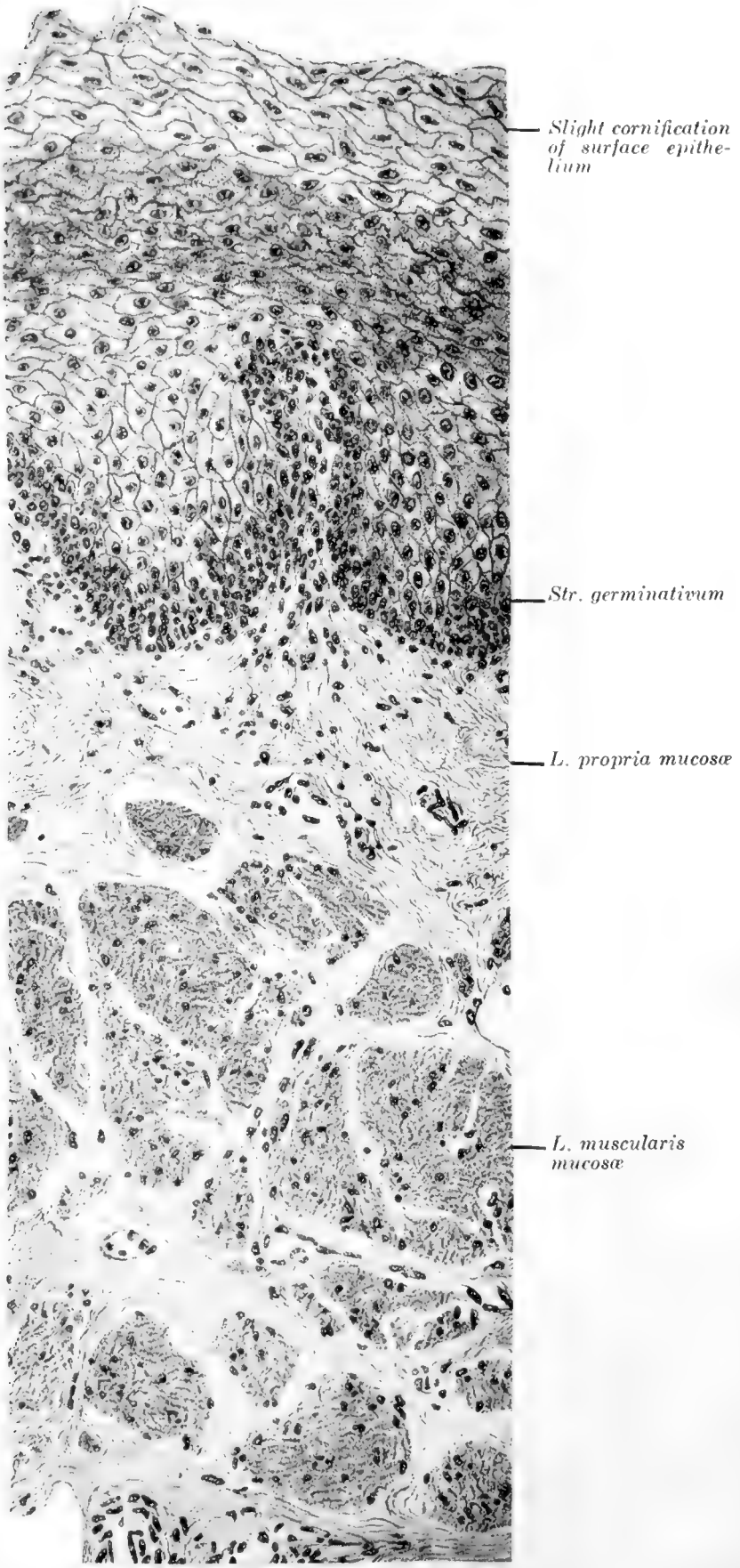

FIG. 13. Transverse section of tunica mucosa of human œsophagus. Ocular 3.

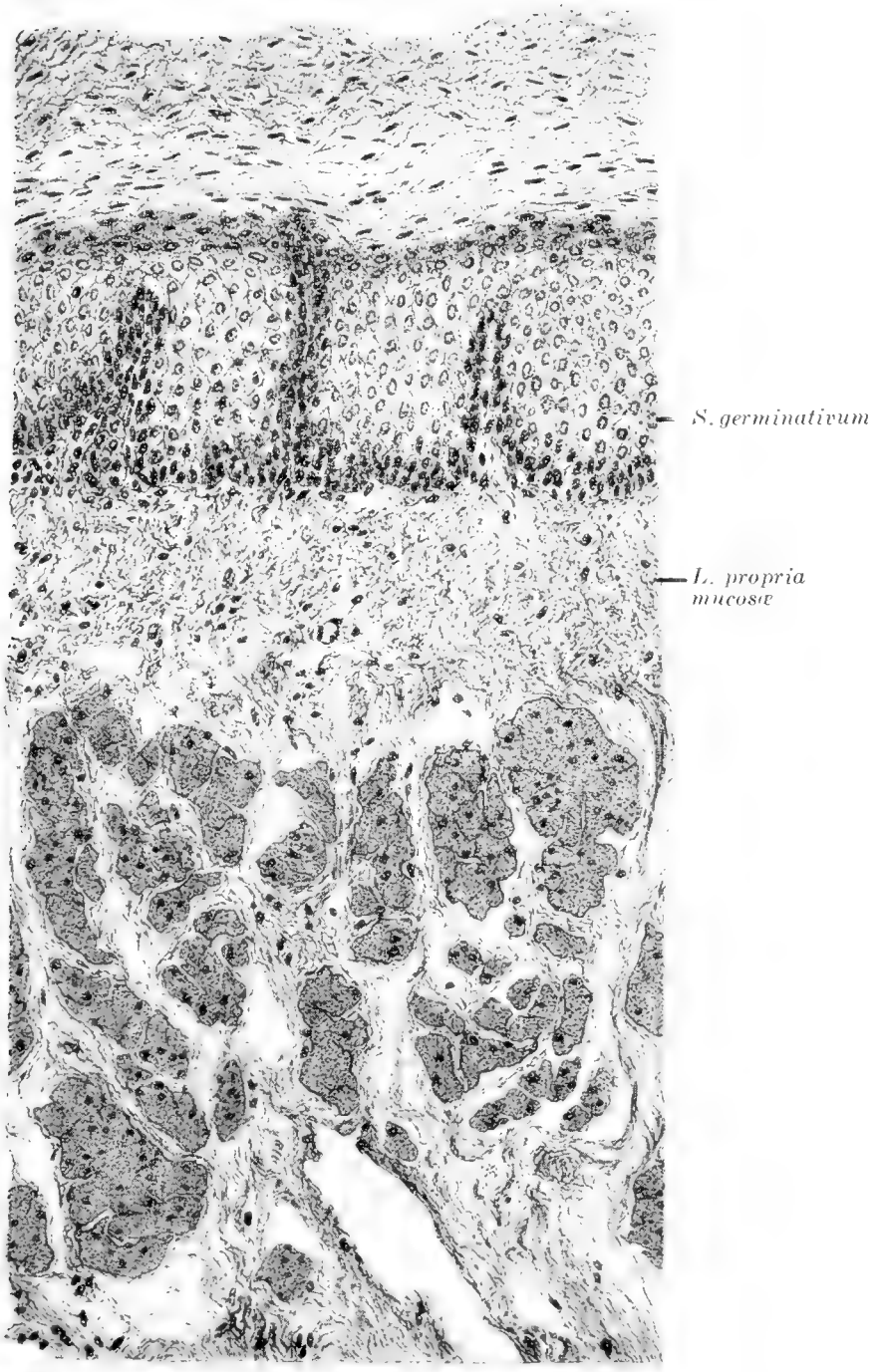

Fig. 14. Transverse section of tunica mucosa of œsophagus of sheep. × 120.

arranged unstriated muscle fibres, and rapidly becomes a complete layer at the upper end of the œsophagus, attaining in the adult a thickness of 0.4 mm.

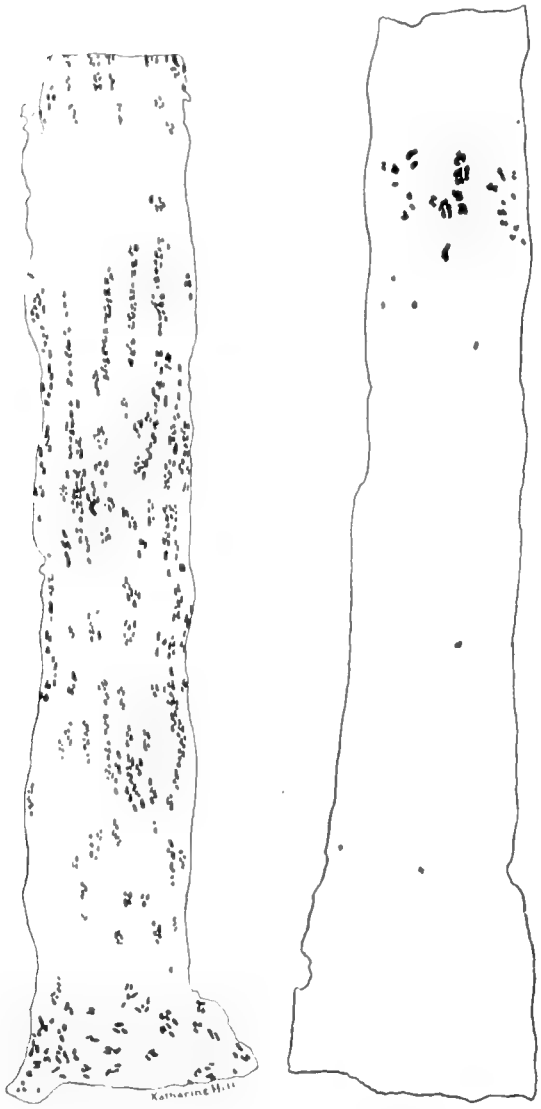

Fig. 15.

Fig. 16.

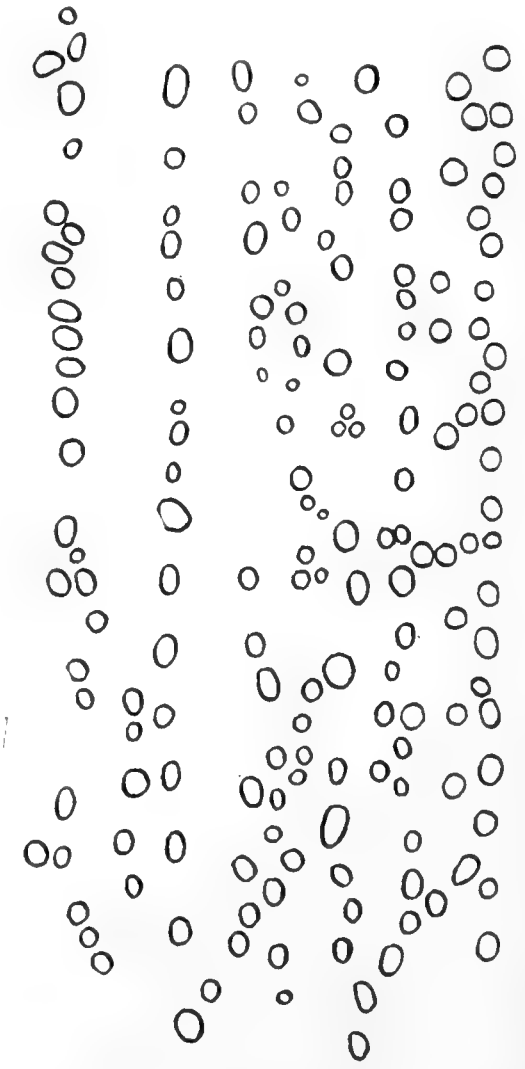

Fig. 17.

Figs 15 and 16. Diagrams showing exact location and relative sizes of every glandular lobule in two human œsophagi. Figures are reduced one-half. The figures represent the œsophagus as seen when cut open along the posterior border and spread out.

Fig. 17. Diagram showing exact distribution of papillæ on a small area of human œsophagus. The rows are parallel with the œsophageal axis.

As regards the character of the tunica muscularis my observations agree in the main with those of Welcker and Schweigger-Seidel

(61) except that I did not find the striated fibres extending so far down. The following table shows the condition found on the posterior wall of two human œsophagi.

No.	Length.	Striated.	Mixed.	Unstriated.
1.	Longit. 21 cm.	6.2 cm.	1.95 cm.	12.35cm.
	Circ.	3.52 cm.	4.63 cm.	12.35cm.
2.	Longit. 21.3 cm.	5.6 cm.	2.4 cm.	13.3 cm.
	Circ.	3.6 cm.	4.75 cm.	12.95 cm.

These measurements show that the unstriated fibers extend somewhat higher in the circular coat than in the longitudinal coat, but that scattered striated fibers extend to about the same level in both coats. Approximately the lower two-thirds of the œsophagus is provided with smooth fibres only. I did not find the striated fibres in the lower end of the œsophagus which have been described by Gillette (72) and Coakley (92). As Schaffer has shown, the unstriated fibres may occasionally extend in the circular coat as far as the lower border of the pharynx or even into the pharynx.

General Conclusions.

In addition to the specific data recorded in the preceding pages certain general conclusions may be drawn from the observations and from those of previous workers.

As regards the occurrence of œsophageal glands the species examined and those previously described may be divided into three groups:

A. Mammals in which the œsophageal glands are wholly lacking below the level of the cricoid cartilage. To this group belong all the rodents examined, including the following species:—Arctomys monax, Tamias striatus, Cavia, Erethizon dorsatus, Lepus cuniculus and Lepus nuttalli, Mus decumanus, Sciurus hudsonicus, and Geomys bursarius. In addition to these Sciurus vulgaris and Spermophilus citillus have been reported by Oppel as lacking œsophageal glands. Here also belong the ungulate species Bos taurus, Ovis aries and Equus caballus, the carnivor species Felis domestica, Vespertilio

fuscus belonging to the Chiroptera and according to Oppel the marsupial species, Trichosurus vulpecula, Aepyprymnus rufescens, and Phascolarctus cinereus.

B. Mammals in which the glands are few in number. To this group belong Erinaceus, Scalops and Lutreola in which a few mucous glands with demilunes occur in the upper end of the œsophagus, and Mephitis in which a few glands of similar nature are to be found at the lower end of the œsophagus.

C. To the third class belong a small group of mammals belonging to different orders in which the glands are present in considerable numbers. These are Didelphys, Procyon, Canis familiaris and C. vulpes. Meles taxus and Nasua rufa, in which glands occur in considerable numbers throughout the whole extent of the œsophagus, Sus scrofa domestica in which they are abundant in the upper half of the œsophagus and few in number in the lower half.

In structure the œsophageal glands are compound tubulo-alveolar glands, consisting, except in those of man, of two kinds of cells, mucous cells and serous cells. The latter elements, which have been overlooked by previous observers with the exception of Klein (79), Renaut (97) and Helm (07) are aggregated in the form of demilunes such as we find in the submaxillary gland of the dog, and like the demilunes of the salivary glands are provided with well defined intercellular secretion canaliculi. In the glands of the lower end of the œsophagi of Procyon and Didelphys these serous cells are so numerous that in many cases they form sessile alveoli on the sides of the mucous tubules with an independent lumen from which the secretion-canaliculi branch off. In man no demilunes could be found, thus confirming the observations of Schaffer.

A survey of the distribution of glands makes it apparent that the occurrence of œsophageal glands is the exception rather than the rule in mammals and that their presence bears no relation whatever to the mechanical qualities of the food nor to the completeness of mastication. Purely vegetable feeders are uniformly devoid of glands in the œsophagus and in many flesh feeders they are either few in number or wholly absent. Ranvier it is true explains the absence of glands in rodents on the basis of the thoroughness of mastication,

but if we examine the matter more closely we find that as a matter of fact the œsophagus of these animals has been specialized on account of the coarse character of the food but in other ways than by the production of mucous glands. On the other hand the theory that the glands are developed in proportion to the needs of the animal for lubrication of the food bolus does not adequately explain the fact that in many Carnivora glands are few in number or wholly wanting while in others they are very abundant. If we examine the cases of those animals which have a very large number of œsophageal glands we find that there is no common quality as regards efficiency of mastication relative to development of the salivary buccal and œsophageal glands, or consistence of the food which would serve to explain their presence. They are, however, without exception mixed feeders, and this suggests that it is the composition of the food rather than its consistence which has called forth the development of mucous glands in the œsophagus, or to express it differently, that the secretion of these glands has a chemical value rather than a mechanical one. This view is supported by the twofold cellular character of the glands, for it is difficult to understand why a serous gland should have been developed where the need was simply the mechanical need of lubrication.

In contrast to the glands the epithelium shows a very definite and constant relation to the character of the food, and it is in the epithelium that we find the œsophagus undergoing specialization in those animals which live on coarse vegetable food. The structure of the epithelium is thus an accurate index of the character of the food swallowed, inasmuch as we find a thickened and highly cornified epithelium in those animals which live on coarse food, particularly vegetable feeders, and a thin slightly cornified epithelium in animals living on soft food, for example, the carnivors.

Correlated with this thickening of the epithelium we find, as might be expected, an increase in the development of the muscularis mucosæ, the probable function of this layer being to retract the mucous membrane above the descending bolus of food. Conversely, the muscularis mucosæ and the epithelium exhibit these specializations in a less degree in those animals in which large numbers of

mucous glands are present, indicating that, although the glands have been developed primarily for another purpose, their secretion nevertheless serves the purpose of lubrication where it is present, and in so doing modifies the degree of specialization of the epithelium and muscularis mucosæ. For example in the pig, in the upper end of the œsophagus where glands are abundant, no muscularis mucosæ is to be found, while in the lower end where few glands are present, it is highly developed.

It will be noted that with regard to the processes of connective tissue which project into the epithelium and which are usually described as papillæ of the lamina propria mucosæ the observations here recorded confirm in general the conclusions of Strahl (89), who found that in many cases they were not true papillæ but elongated ridges of the lamina propria running in a direction parallel to the long axis of the œsophagus and connected with one another by oblique ridges. This is a matter in which it is very easy to be mistaken because ridges cut across are very similar in appearance to papillæ. It is only by examining carefully series of sections, or whole preparations of the epithelium, that this mistake can be avoided. Among the mammals examined true papillæ were found in the pig, ox, horse, sheep, and man and were associated in all these cases with longitudinal ridges of the lamina propria. In Didelphys, Arctomys, Sciurus, Tamias, Lepus, Canis, ridges only were present, although in Cavia and Tamias the irregular summits of the ridges afford suggestion of beginning papillæ. In Procyon there are longitudinal ridges also with suggestions of low papillæ on their summits, while in Mus, Geomys and Erethizon, there are neither ridges nor papillæ, but an epithelium of fairly uniform thickness. In Mephitis, finally, we have represented the exact opposite of connective tissue papillæ, inasmuch as the epithelium sends processes into the subjacent connective tissue. This is of course to be derived from a further development of the system of longitudinal and transverse ridges which have become so numerous and close that they surround papilla-like processes from the deep surface of the epithelium.

With regard to the phylogeny of the œsophageal glands, the fact that in whole orders no glands are to be found and that each of the

principal orders both of marsupials and placentals contain individuals wholly lacking in œsophageal glands indicates that these structures have been developed independently in the different orders in response to needs that have arisen as result of food specialization. The alternative hypothesis that they were originally present in all mammals and that they have disappeared from some is scarcely worth consideration in view of the general absence of similar glands in lower vertebrates.

In man alone is there any indication that the œsophageal glands are disappearing. Here the great variability in number of the glands and the constant presence of cyst formation and stasis of the secretion and atrophy of the glandular cells might be taken as an indication of a disappearing structure.

In conclusion I desire to express my thanks to Prof. R. R. Bensley for valuable suggestions during the course of this investigation. I desire also to thank Miss Katharine Hill for the accompanying drawings.

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THE LIMIT BETWEEN ECTODERM AND ENTODERM IN THE MOUTH, AND THE ORIGIN OF TASTE BUDS.[1]

I. Amphibians.

BY

J. B. JOHNSTON.

With 21 Text Figures.

It has been generally recognized that in man it is not possible to locate exactly the limit between ectoderm and entoderm after the rupture and disappearance of the pharyngeal membrane. At the same time it is clear that the tongue arises from the lower ends of the visceral arches and that the floor of the mouth is in the greater part lined by entoderm. In the roof the facts are not so clear, but it is believed that the stomodæum is much deeper above than below. Obviously it can not include the eustachian tube nor the nasal cavity, although the latter is of ectodermal origin. How much of the maxillary process and the palatal shelf is covered by ectoderm, and how much by entoderm, is not known.

The taste buds are all located in the pharyngeal portion or floor of the mouth, with the exception of those in the soft palate. Whether the soft palate is covered by ectoderm or entoderm remains to be determined. In view of the fact that at least the great majority of the taste buds are found in the area which all authors agree is lined by entoderm in the embryo, the question may be asked what reason, if any, is there for considering that the taste buds are *not* of entodermal origin? The chief reason is the general doctrine that nervous structures are of ectodermal origin. The history of that doctrine can not be traced here, but it may be said that it does not appear to be based upon an exhaustive study of the origin of all

[1]Neurological Studies from the Institute of Anatomy, University of Minnesota, No. 7.

The American Journal of Anatomy.—Vol. 10, No. 1, Jan., 1910.

nervous structures. The origin of certain visceral plexuses in man and of various peripheral nerve cells in other vertebrates has not been determined, and the origin of taste buds is at present under discussion. The statement that *all* nervous structures are derived from the ectodermal layer of the embryo is not warranted by the known facts. Another reason for thinking that taste buds are of ectodermal origin is that in certain fishes they are found in the later embryo and adult in ectodermal territory, namely, the outer skin.

In 1898 I made the statement that in the head of vertebrates "all sensory structures of ectodermal origin are supplied by components of the Vth (including spinal Vth components running in other nerves), VIIIth and lateral line nerves, and that all fibres supplying such structures have their central endings in the nucleus funiculi, tuberculum acusticum or cerebellum. . . . On the other hand, all sensory structures of entodermal origin are supplied by VIIth, IXth and Xth components, and all fibers supplying such structures have their central endings in the lobus vagi."

The inclusion of taste buds among structures of entodermal origin was adversely criticised by Strong (1898), and it did not appear that any neurologist was ready to entertain the idea that taste buds were possibly of entodermal origin. I have since brought forward evidence (1905) that in petromyzonts the taste buds arise in entodermal area. In the ammocœtes stage I was able to find them only in entodermal surfaces. In teleosts (Coregonus, Catostomus) the buds first appear in the pharynx and œsophagus where there seems to be no possibility of origin from any other source than entoderm. The appearance of taste buds in entodermal area in teleosts has since been confirmed by Landacre (1907), working on Ameiurus. He recognized, however, the possibility of their being formed in ectoderm also.

Material and Methods.

I have undertaken to study the limits of ectoderm and entoderm in the mouth of several vertebrates, both for its general interest and with especial reference to the origin of taste buds.

Amphibians offer especial advantages for the study of the relations

of ectoderm and entoderm owing to the persistence of yolk in the entoderm cells after it has disappeared from the ectoderm. The early development of the mouth has been studied fully in Amblystoma punctatum and the following description applies wholly to this form. Necturus and frog embryos have been studied sufficiently to control the chief facts.

While at West Virginia University I made a large collection of Amblyostoma embryos and larvæ from the unsegmented egg to tadpoles over an inch in length (about March 20 to July 1). Various methods were tried both for fixation and staining. The fixing fluid that gave the best results was the formol-sublimate-acetic mixture devised by Worcester.[2] All the embryos used for this study were fixed in that fluid. For staining, borax carmine, hæmalum, hæmatoxylin with counter stains have been used. Sections prepared for this particular purpose have all been stained by iron hæmatoxylin and counter stained by acid fuchsin. It was found that iron hæmatoxylin stains yolk granules more intensely than any other element in the tissues and remains in the yolk granules in the differentiating bath until it is removed from all other structures, even the chromatin. By differentiating to the last degree favorable for study of chromatin and counter staining with acid fuchsin, clear transparent preparations are obtained in which the yolk granules stand out boldly on a pink ground, while the nuclei and cell boundaries are lightly stained but distinctly seen.

As is well known, the ectoderm and entoderm differ from the earliest stages in the number and size of yolk granules contained in the cells. This difference is readily made out in any sections of well fixed material by careful study. In preparations stained as above described the differences are sharply marked and become more prominent and striking as development proceeds. The yolk granules in the ectoderm grow smaller and less numerous and disappear at a relatively early period. In the brain the yolk persists longer than in the ectoderm, but in such small granules that there is no comparison with the entoderm. The muscle-forming masses and some parts

[2]Formula: take 10 per cent formol, saturate with sublimate, add glacial acetic acid to make 10 per cent of the whole.

of the mesenchyme retain large quantities of yolk, but these do not enter in any way into the structures to be studied in this connection. The ectoderm and entoderm are drawn as faithfully as possible in the figures. When it is remembered that the general gray shading of the cells represents fuchsin stain, while only the granules and chromatin are black, it will be seen that the distinction between ectoderm and entoderm is many times more striking in the preparations than it is in the figures. In late stages, when the yolk begins to be used up in the entoderm, the sharp staining of the granules is of the greatest advantage. Before that time the yolk has entirely disappeared from the ectoderm and most of it from the nervous system.

In such a study as this good serial sections without tearing or distortion are necessary, and the yolk itself is well known as a great obstacle to obtaining such sections. I have tried some methods of fixation in which the yolk is said to be made soft and easily cut. I have not found, however, that such embryos show such perfect fixation as I desired for the study of the early stages in the formation of taste buds. The fixing fluid used renders the yolk hard and brittle, but fixes faithfully all elements of the tissues and without any distortion or shrinking. To obtain perfect serial sections in the three planes I used the method of imbedding in a paraffin-rubber mixture made as follows: to 100 cc. of paraffin which melts at 1 or 2 degrees higher temperature than desired in the imbedding mass, add 1 to 2 grams of crude India rubber cut in as small bits or shreds as possible. Heat over boiling water 24 hours or let stand in the bath at 60° C. for three days. Pour off the clear melted paraffin and keep in the solid state. Use exactly as paraffin, clearing specimens for imbedding in xylol or toluol. With this method I have obtained many series of sections of all stages of Amblystoma as perfect as it is usual to obtain from ordinary objects. Obviously I could not use for this study preparations in which the yolk-bearing tissues were torn, or in which the yolk granules were scraped out of their position and driven along by the knife. In the figures given every yolk granule is faithfully drawn under the camera as accurately as possible in its relations to the cell boundaries and nuclei. No yolk granule is omitted because it seems to be displaced. No drawings

are taken from sections which show the slightest tearing or breaking in the parts concerned.

1. *Early Form and Relations of Entoderm.*

After completion of gastrulation the archenteron has the well known form characteristic of amphibian embryos. The enlarged anterior part of the archenteron is bounded in the region where the mouth will form by a relatively thin layer of entoderm and a thinner layer of ectoderm (Fig. 1). In this region are two broad, very shallow depressions in the ectoderm. The more anterior one represents the hypophysis, the posterior one the future mouth. Opposite these the archenteron itself presents two prominent pits (or angles) as seen in sagittal sections. The anterior one is a slender pointed

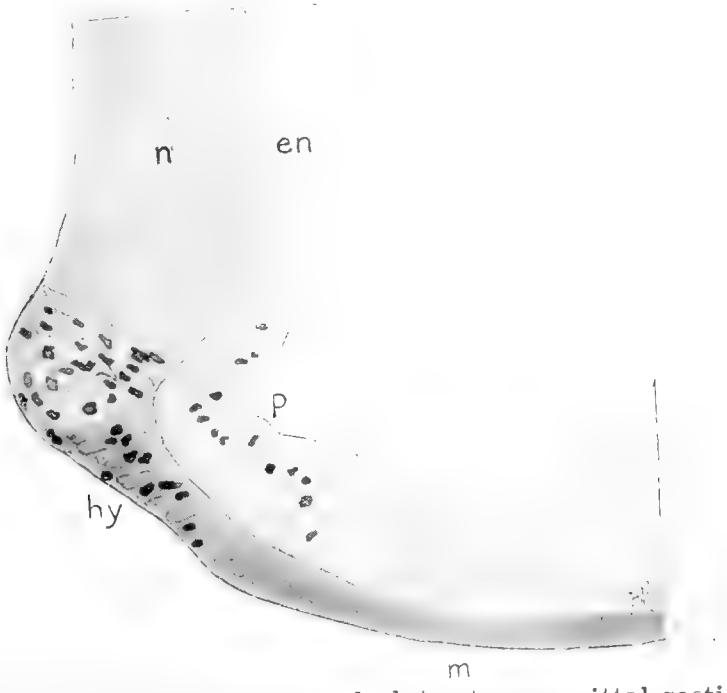

FIG. 1. Amblystoma punctatum, neural plate stage, sagittal section of mouth region. Borax carmine stain.

cavity (Fig. 2), and the entoderm surrounding it forms a blunt wedge projecting between the neural plate and the ectoderm. The posterior one is a broad depression which corresponds to the future mouth opening. The entoderm surrounding the anterior pit is the preoral entoderm and corresponds in every way to the preoral entoderm of most selachians (cf. Johnston, 1909).

When the neural plate rolls up into a tube (Fig. 2) pressure is exerted upon the archenteron by the brain portion of the neural tube and the effect is seen in a compression of the archenteric space. The two angles seen in sagittal sections are now more marked and are separated by a thickening or fold of the intervening entoderm. In the meantime the notochord and mesoderm are forming in the same manner as in selachians. In the stages represented in Figs. 2 and 3 the notochord ends anteriorly in a median mass which is not separated from the entoderm and is continuous laterally with the mesoderm which is in process of splitting off from the entoderm. This median mass remains in continuity with the preoral entoderm.

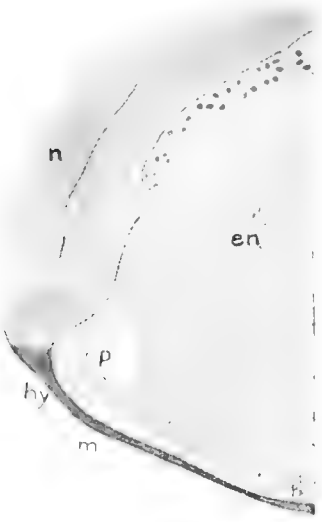

Fig. 2.

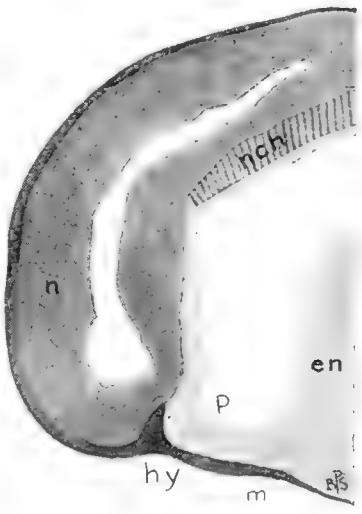

Fig. 3.

Fig. 2. Amblystoma punctatum, neuropore stage. The foregut is already compressed and the palæstomal (*p*) and neostomal recesses are sharply marked. Borax carmine.

Fig. 3. A. punctatum, after closure of neuropore. Borax carmine.

I have indicated in an earlier paper (1903) that the mesoderm formation is somewhat delayed in the head of Amblystoma so that the mesoderm of the hyoid and mandibular arches is split off from the entoderm as separate rods of mesoderm. The median mass mentioned is continuous laterally with the mesoderm which forms the premandibular somite (Fig. 4, A and B). The splitting off of the notochord stops at the posterior border of this median mass and in later embryos the point at which the end of the notochord remained

longest in connection with the entoderm is marked by a sharp notch (see Johnston, 1906, Fig. 36). In front of this the entoderm is not thick as in selachians so that the term "median mass" is not so applicable. However, from this part of the entoderm mesenchyme splits off and leaves the entoderm very thin, often scarcely a continuous membrane. This thin area is soon filled up by the shortening and

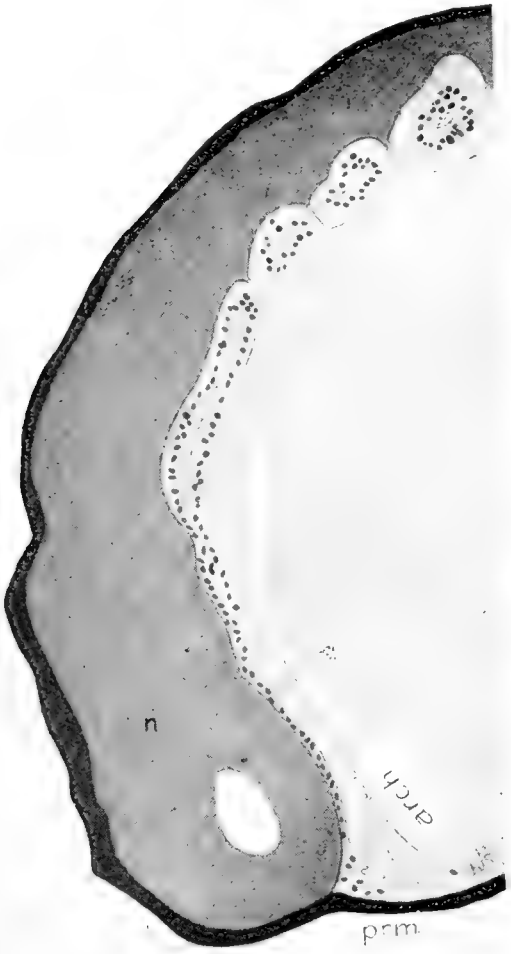

Fig. 4. A

Fig. 4. B

Fig. 4. A. punctatum, two parasagittal sections to show the relations of the head mesoderm. Hæmalum.

shifting back of the preoral entoderm and by the general compression which soon obliterates the anterior end of the archenteric cavity.

2. *Development of Hypophysis.*

The ectoderm which will form the hypophysis can be recognized as soon as the neural plate is formed (Figs. 1, 2, 3). Below the

terminal ridge of the neural plate (see Johnston, 1909) the ectoderm grows thinner. The wedge-shaped piece of ectoderm immediately adjacent to the neural plate will form the hypophysis. Its position is accurately indicated in Figs. 1 and 2. When the preoral entoderm is more blunt or rounded the hypophysis presents in the earliest stages a triangular form in sagittal sections (Fig. 3), one angle being directed inward between the terminal ridge of the neural plate and the preoral entoderm. When the neural plate rolls up the terminal ridge participates in the rising up of the neural folds, and

Fig. 5. A. punctatum, stage when hypophysis begins active invagination. Median sagittal section. Hæmalum.

the distinction between the neural plate and hypophysis at once becomes clearly marked (Figs. 2 and 3). From the earliest stage the hypophysis rests against the tip of the preoral entoderm.

While the neural tube is rolling up, the hypophysial ectoderm becomes deeper, so that in sagittal section the depth of the triangle becomes greater than the length of its base on the surface (Fig. 3). Up to this time the cells within the triangle show no regularity of arrangement. Immediately following this it is seen that the deeper cells are arranged so as to suggest a short blind sac. From the start (Fig. 5) this sac is thicker on the anterior or dorsal side. It has

no actual cavity, but the arrangement and pigmentation of the cells suggest a cavity, and the wall next to the entoderm is thin and devoid of nuclei. By this time the ectoderm of the mouth plate, caudal to the hypophysis, consists of only a single layer of flat cells (see

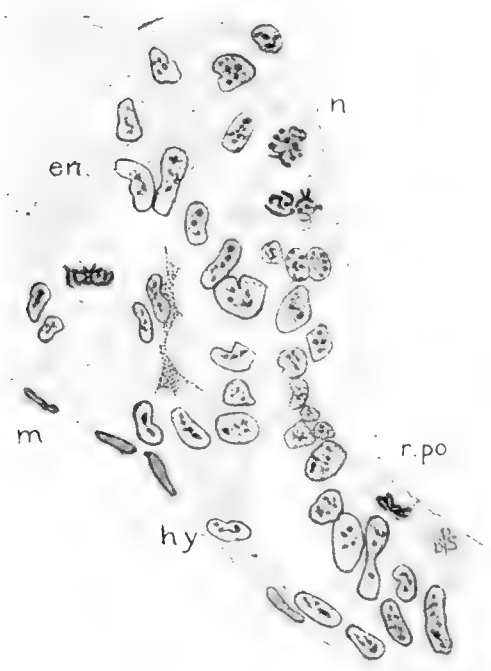

Fig. 6.

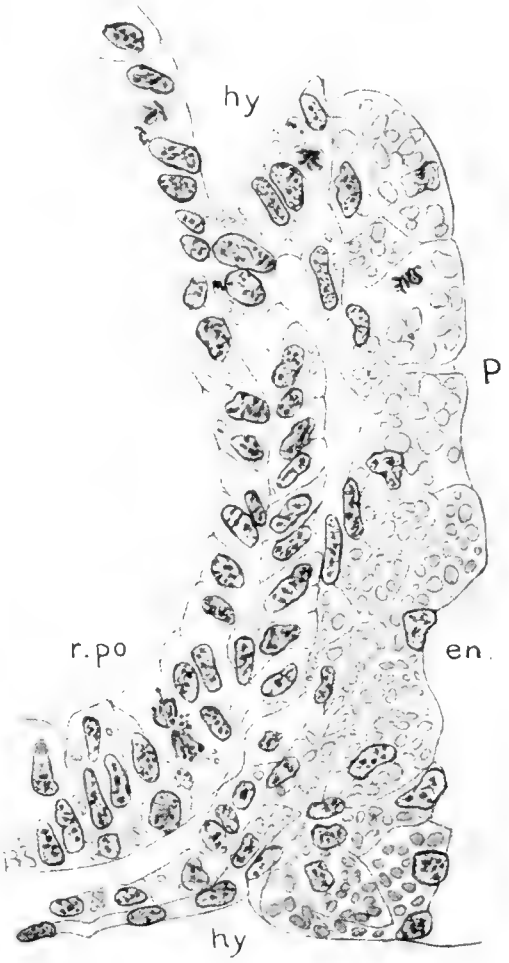

Fig. 7.

Fig. 6. A. punctatum, in a stage slightly advanced over that in Fig. 5. Medial sagittal section of hypophysis. Borax carmine.

Fig. 7. A. punctatum, at the height of the hypophysial invagination before separation from ectoderm. Note the concavity of hypophysis and the absence of ectoderm from the mouth plate. Hæmalum.

below). As the hypophysis pushes in, its thin posterior or ventral wall is devoid of cells, and soon the cavity of the hypophysial invagination is bounded on that side by entoderm (Figs. 6 and 7). The hypophysis appears as a hook-shaped membrane (sagittal section)

extending in from the deeper layer of the ectoderm. A slight cavity is contained within the recurved wall at the deeper end (Fig. 7), the rest of the ventral wall being formed by entoderm.

While the hypophysis is pushing in, the preoral entoderm is pushed back, flattened, and its cavity nearly obliterated. This cavity becomes less prominent before the closing up of the entodermal mouth

FIG. 8. A. punctatum, nearly median sagittal section to show the persistence of the preoral or palæostomal recess in the entoderm. The invagination of the hypophysis is very tardy in this specimen. Hæmalum.

pit. There is always to be seen, however, a shallow pit or an angular prolongation of the archenteron which continues to indicate the preoral cavity (Figs. 8, 9, 10). In some cases a cleft appears in the entoderm leading toward the cavity of the hypophysis. By this time the entoderm has been compressed and thickened, but the per-

sistence of the preoral cavity (in various degrees) serves to mark the position of the preoral entoderm. It also explains the peculiar form taken by the hypophysis. The cleft meeting the cavity of the hypophysis suggests that the intact wall of the hypophysial invagination constitutes, with the entoderm dorsal to the cleft, the dorsal wall or roof of the palæostoma, while the absence of a ventral wall to the hypophysial invagination allows the hypophysis to open into

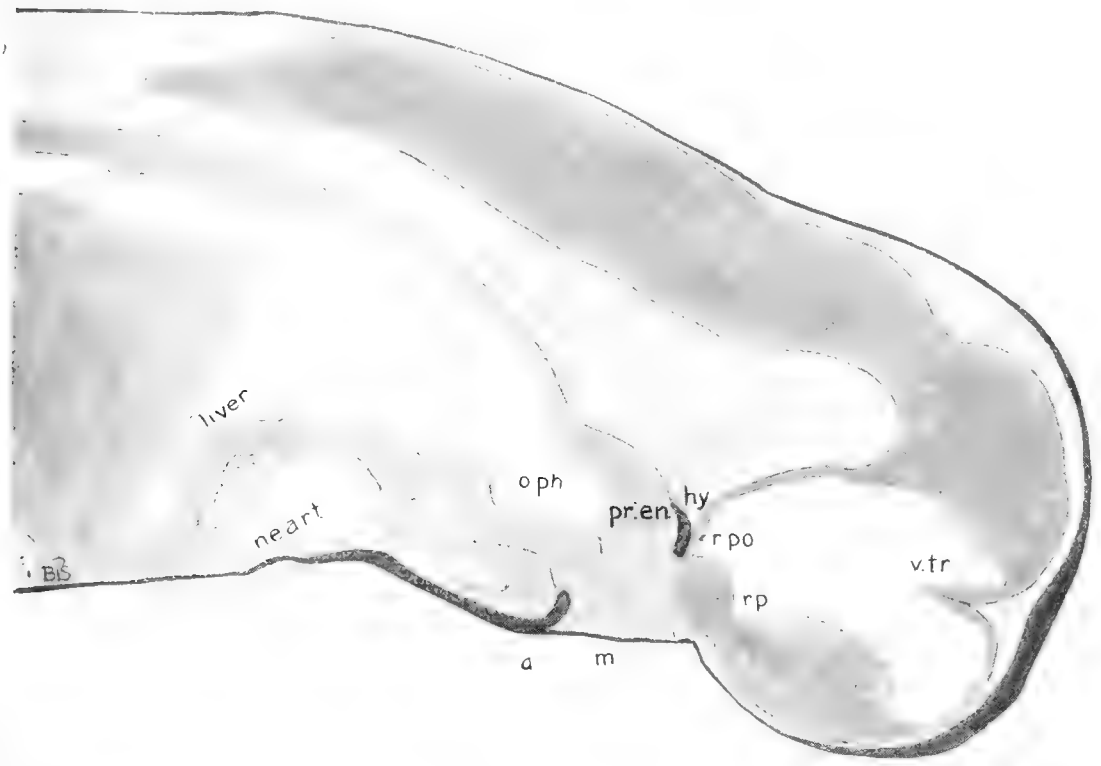

Fig. 9. A. punctatum, after separation of hypophysis from ectoderm. Medial sagittal section. The pointed recess of the archenteron directed toward the letter *m* is the oral recess; the slight pit or concavity next to *pr. en.* is the palæostomal recess. Hæmalum.

the archenteron, the ventral wall or floor of the palæostoma being formed by entoderm alone. These relations are indicated in a diagram (Fig. 11).

After the stage at which the partial connection between hypophysis and archenteron is seen, there is a further bending of the brain, the flexure becoming excessive in amphibians. The bending, together with the expansion of the forebrain, results in pushing the hypophysis

and preoral entoderm farther and farther back, until they come to lie close to the end of the notochord. Compare Figs. 3 and 10. It is this movement of the preoral entoderm dorso-caudally that was referred to above as the means of thickening up the wall of the archenteron which is left so thin by the splitting off of the head mesenchyme. The hypophysis finally comes to lie on the dorsal surface of the preoral entoderm, and the notch which represents the original preoral cavity is visible up to the stage when the velum transversum, epiphysis, paraphysis and the inferior lobes of the brain are all well formed (Fig. 10). The formation of the saccus vasculosus

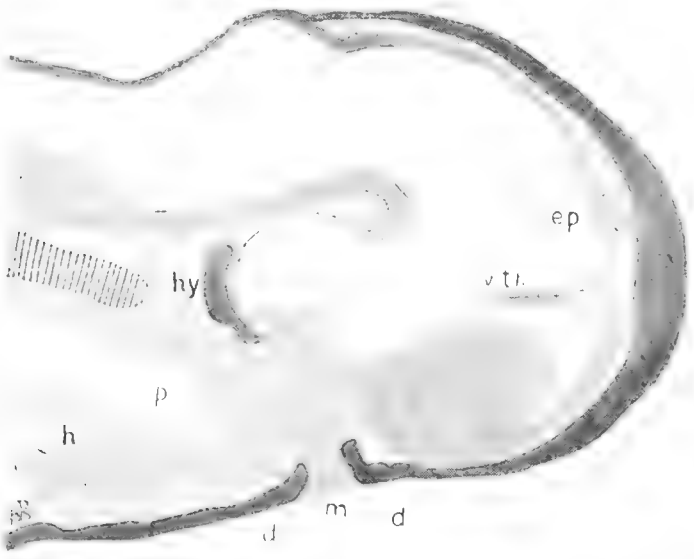

Fig. 10. A. punctatum, stage after formation of epiphysis. Median sagittal section. The palæostomal recess is a deep angular pit. The dental ridges are growing in. Hæmalum.

and the differentiation of other structures in the region of the inferior lobes takes place considerably later.

The shifting caudad of the preoral entoderm and hypophysis which I have attributed to the brain flexure is accompanied by a very considerable elongation of the mouth entoderm. While the shifting is taking place the mouth entoderm is compressed into a solid mass, and this becomes elongated into a comparatively slender cord, flattened dorso-ventrally (Figs. 10, 12, 14, 16).

For some time after its separation from the ectoderm the hypophysis continues to have the form of a shallow cup with the convexity

against the brain, the concavity toward the entoderm (Fig. 9). It finally forms a somewhat ovoid mass and lies beneath the brain. Its further history does not concern us in this study.

3. *Coalescence of the Walls of the Foregut.*

It has already been indicated that the formation of the neural tube begins to compress the anterior part of the archenteron. This is

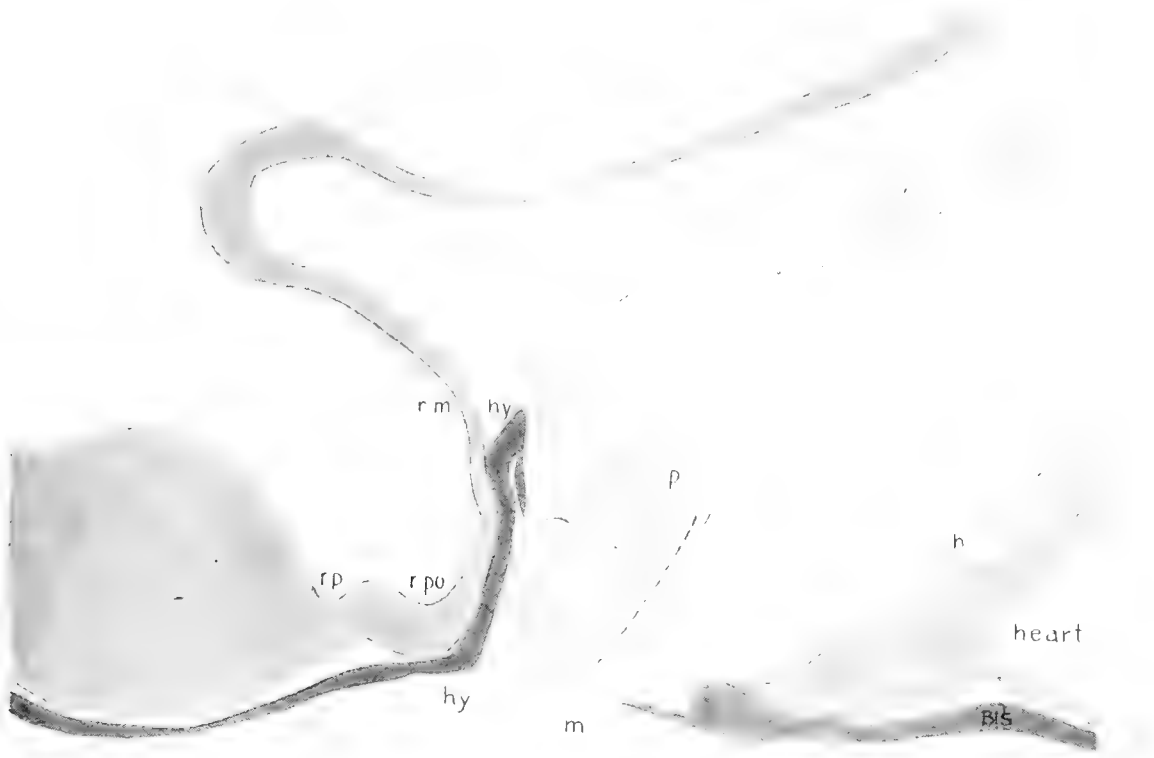

Fig. 11. A diagram of the relations of the palæostoma and neostoma in A. punctatum. A camera drawing was made from the section from which Fig. 7 was drawn and the cleft connecting the archenteron with the hypophysis was made wider. This is the only diagrammatic feature of the drawing. The palæostoma is represented by the cleft *hy- p*.

carried further by the formation of the mesodermic somites above and at the sides and of the heart below, and the bending down of the brain completes the influences which result in the complete obliteration of the cavity of the anterior part of the future mouth (Figs. 12, 14). This condition is well known in amphibian embryos and requires little comment. Two things should be noted. (*a*) The coalescence of the walls begins early and is followed by a relatively

great elongation of the pharyngeal region. The necessity of this to make room for the branchial apparatus, heart and brain, is clear from Figs. 3, 9 and 10, and it is this chiefly which causes the forward projection of the head from the yolk and the straightening out of the embryo. (*b*) When the oral cavity appears it leads to the place which has been indicated in the early embryo as the future mouth opening. The history of the hypophysis and preoral entoderm given above and the later history of the preoral entoderm make this clear.

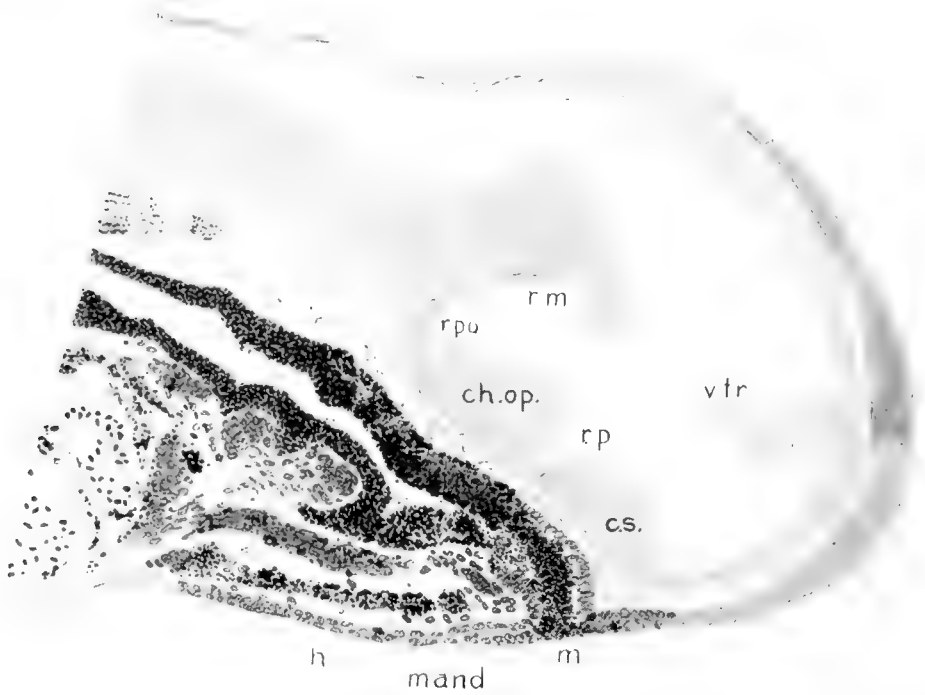

Fig. 12. A. punctatum, nearly median sagittal section of oropharyngeal region after the coalescence of the walls of the primitive mouth cavity. The yolk-laden entoderm is surrounded by the ingrowing dental ridges. Iron hæmatoxylin, fuchsin.

4. *Disappearance of Mouth Plate Ectoderm.*

In Amblystoma no stomodæum is formed. Instead, the ectoderm over the mouth plate area disappears, leaving the solid entoderm exposed on the free surface. Later the mouth cavity appears as a cleft in this entoderm.

The well known arrangement of the cells of the ectoderm in two layers is clearly marked in the mouth plate area of Amblystoma in the neural plate stage. As the neural tube rolls up the ectoderm

becomes somewhat thinner, but not as thin as that covering the ventral surface of the yolk mass. Following this the inner cells of ectoderm disappear from the mouth plate. In either transverse or sagittal sections the entoderm is seen to be covered by the thin flat cells of the outer layer of ectoderm, while the inner layer of more cuboidal cells stops abruptly around the borders of the mouth plate (Figs. 5, 6, 13). Anteriorly the mouth plate is bounded by the hypophysis. I have not been able thus far to see in detail how this

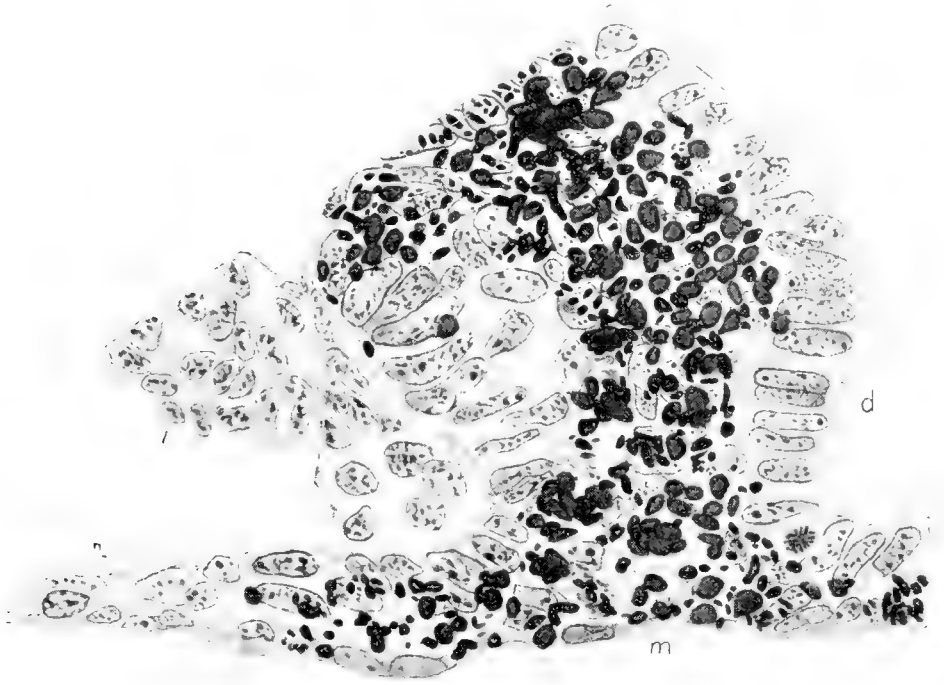

Fig. 13. Detail drawing of the mouth plate and dental ridges in the section drawn in Fig. 12. Here it is seen that the outer layer of ectoderm is partly broken down but still covers the entoderm.

condition comes about. Whether the inner cells of ectoderm migrate apart and draw away from the mouth area or whether some of these cells disintegrate in situ is not wholly clear. I have seen no signs of disintegrating cells, and there is to be seen a heaping up or thickening of the inner layer of ectoderm around the mouth plate. I am strongly inclined to think that the inner cells draw away from the mouth plate in preparation for the formation of the dental ridges to be described below. Certain it is that by the time the hypophysis begins to be actively invaginated (Fig. 6) the ectoderm of the mouth

plate consists of the thin outer layer alone. As development goes on this thin outer layer gradually breaks down and its cells seem to scale off, leaving the yolk-laden entoderm cells freely exposed (Figs. 7, 8, 9, 10, 12, 13, 14, 15).

This disappearance of the mouth plate ectoderm I consider comparable to the rupturing of the pharyngeal membrane so far as its ectodermal portion is concerned. The entodermal layer of the membrane is not ruptured at this time because of the mechanical condi-

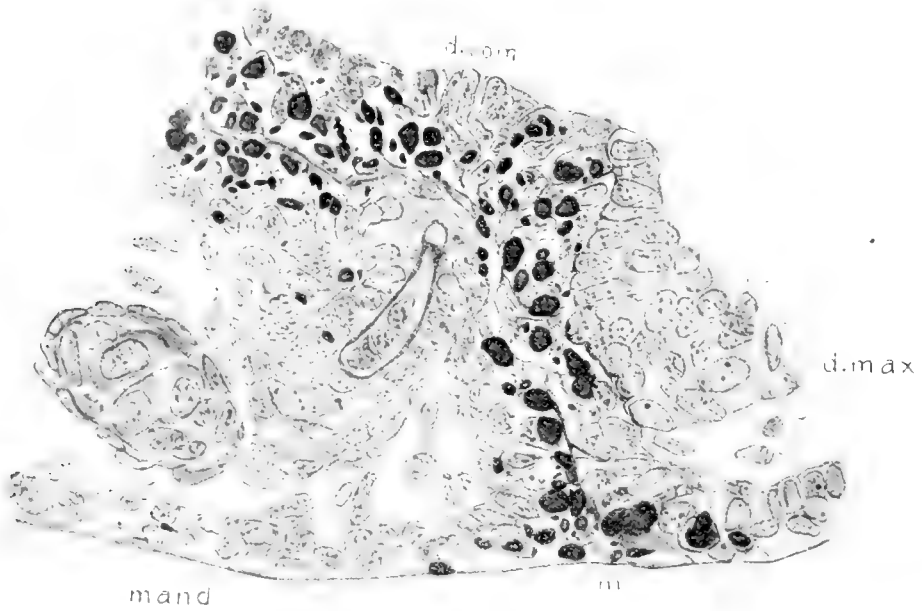

FIG. 14. A. punctatum, stage of tooth formation. Median sagittal section of the mouth region. The oral entoderm is drawn out into a slender flattened rod and the formation of the mouth cleft will soon take place. A mandibular tooth is seen in vertical section and dorsal to the entoderm, the ectodermal in-growth in which the maxillary and vomerine teeth will appear is distinguished by the absence of yolk. Between *d. max.* and *d. vom*, is the projection of entoderm which later meets the nasal sac. Iron hæmatoxylin, fuchsin.

tions which lead to the coalescence of the walls of the archenteron. The appearance of the oro-pharyngeal cavity as a cleft in the entoderm completes the process, which is comparable to the rupturing of the pharyngeal membrane in other vertebrate embryos.

5. *Formation of Dental Ridges and Teeth.*

The thickening of the inner layer of ectoderm surrounding the mouth-plate mentioned above soon becomes prominent, and in both

sagittal and transverse sections it is seen that this layer turns in around the entoderm (Figs. 8, 9, 10, 12, 13). This in-growing ectoderm pushes in farther on the dorsal surface of the entoderm than on the ventral and soon forms a ring or collar around the solid oral entoderm. At first the ring is incomplete in its mid-dorsal portion where the inner layer of ectoderm is taken up in the formation of the hypophysis. The ingrowing ectoderm spreads to fill up this

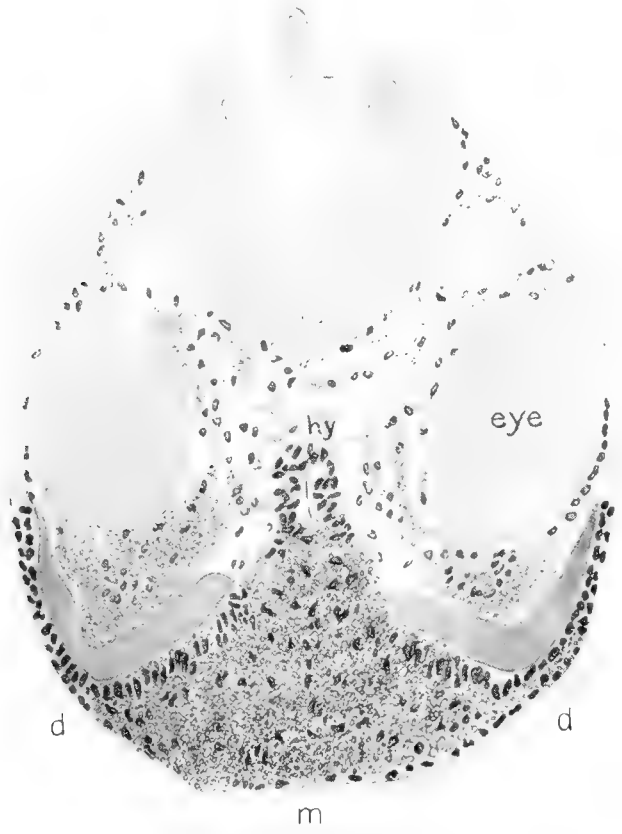

Fig. 15. A. punctatum, formation of dental ridges. A transverse section through the mouth plate after disappearance of the ectoderm. Hæmalum.

gap, and pushes up on the dorsal surface of the oral entoderm to about the point of contact of preoral entoderm and hypophysis. This mid-dorsal portion of the in-growing ectoderm gives rise to the vomerine teeth. The in-growing ectoderm forms the dental ridges in which the teeth soon begin to make their appearance (Figs. 12-18). For the purpose of this study the formation of the teeth need not be followed in detail. One point only is of especial interest here, namely, that when the teeth are formed they are separated from

the mouth cavity by a layer of entoderm. The eruption of the teeth involves the piercing of this entoderm (Figs. 14, 16, 18, 20).

6. *Appearance of Oro-Pharyngeal Cavity.*

The splitting of the solid entoderm takes place progressively from behind forward at a stage after the teeth are well formed in the dental ridges (Figs. 14, 16, 17). By the time the splitting of the oral entoderm takes place the mouth region has grown wider from side

Fig. 16. A. punctatum, appearance of mouth cleft. Median sagittal section of the mouth. The ectoderm and dental ridges are more deeply shaded. The yolk granules are all drawn and the outline between ectoderm and entoderm is taken from the cell boundaries. Iron hæmatoxylin, fuchsin.

to side in anticipation of the future wide mouth. The whole anterior part of the entoderm has taken part in this widening, so that the oral entoderm is now a broad flat solid plate. As the pharyngeal cavity progresses toward the mouth it advances most rapidly toward the lateral angles of the mouth. When the tooth germs begin to form there is a thin place left in the ectoderm between the maxillary and vomerine teeth. At this place the entoderm forms an elbow dorso-cephalad which is quite pronounced at the lateral border of the tooth-forming areas (Figs. 14, 17, 18). In the meantime the

nasal sacs have been formed and the wall of the nasal sac comes into contact with the elbow of entoderm lateral to the maxillary and vomerine teeth. The pharyngeal cleft invades this part of the entoderm first, and a connection is established between the cavity of the nasal sac and the oro-pharyngeal cavity (Fig. 17). In this way the alimentary canal has two openings to the exterior through the nasal sacs for one or two days before the mouth is open. When the mouth cleft is finally completed the entoderm lines the oral

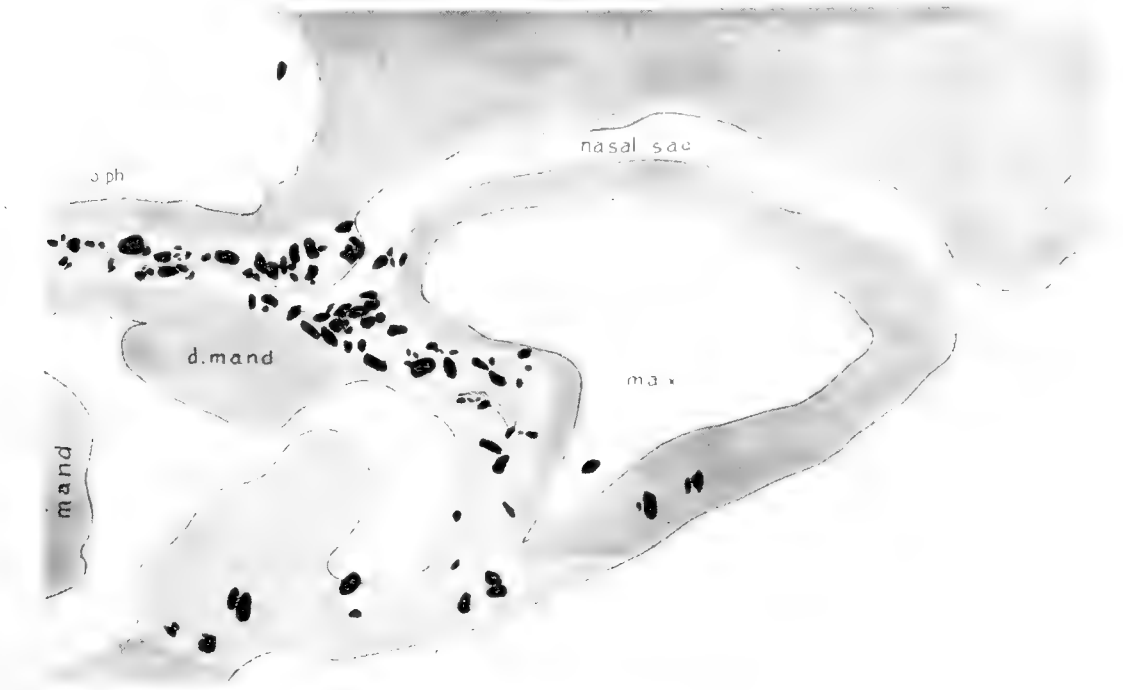

Fig. 17. Parasagittal section from the same series as Fig. 16. The section passes through the opening of the nasal sac into the oral cavity.

cavity to the extreme borders, and even extends out slightly on the surface (Figs. 16, 17). Now the maxillary, mandibular and vomerine teeth pierce the entoderm and enter the mouth cavity.

In the series of embryos used for this study the limits of the entoderm are clearly visible for a period of about ten days after the mouth cleft was formed. (The rate of growth was slow as the running water in the laboratory was colder than that in the ponds.) The entoderm during this period is thicker than the ectoderm, contains numerous yolk granules which are lacking in ectoderm and is more opaque in appearance in sections. The entoderm lines the

whole oro-pharyngeal cavity, meets the ectoderm about midway on the anterior and posterior surfaces of the branchial arches in the gill slits and meets the ectoderm in front of the maxillary and mandibular teeth at the lips.

7. *Formation of Taste Buds.*

The taste buds begin to be formed shortly before the mouth cleft forms. They appear eventually, more or less numerous, on all parts of the floor and roof of the oro-pharyngeal cavity forward to, but not in front of, the maxillary and mandibular teeth. The buds appear

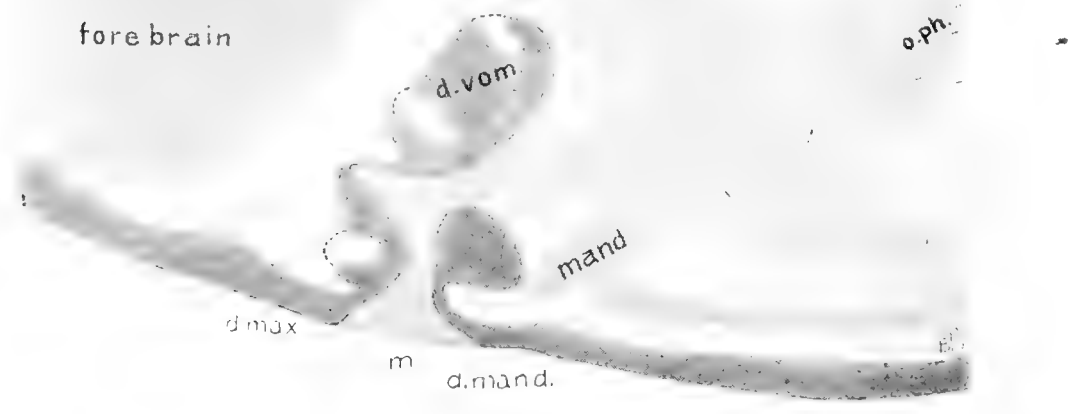

Fig. 18. A. punctatum, stage when the teeth are forming. Median sagittal section of mouth region to show forward projection of entoderm between maxillary and vomerine teeth through which the connection of nasal and oral cavities is formed. Iron hæmatoxylin, fuchsin.

first in the pharynx and later farther forward, as I found to be the case in teleosts (1905). In the larval stage of Petromyzon they are found in the pharynx only. The buds in the greater part of the roof of the mouth and pharynx and those in a large part of the floor are formed in entoderm far removed from any ectoderm. There is, and can be, no question as to their origin from entoderm. The buds which are formed on the gill arches are always on the inner surface, and there is no reason to doubt that they are formed from entoderm cells as are those in the roof of the pharynx far removed from the ectoderm. The buds which are formed in the vicinity of the teeth are the ones which one might think would be derived from the in-

growing ectoderm. The fact that this ectoderm forms dental ridges does not wholly exclude the supposition that some of the cells might give rise to taste buds. Therefore, an account of the mode of formation of taste buds in the front part of the mouth will be sufficient to determine whether any of the taste buds are derived from ectoderm.

Fig. 19 shows a taste bud forming in the floor of the mouth a short distance behind the mandibular teeth at the time when the mouth cleft is forming. The ectoderm is given a darker shade than

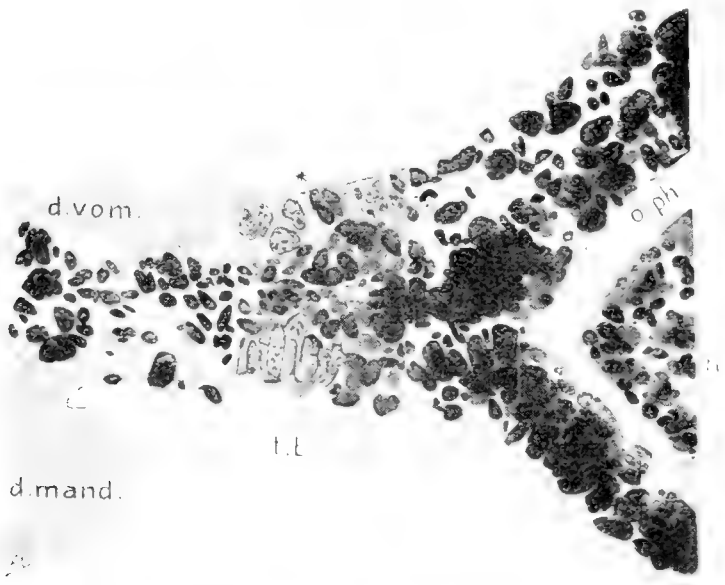

Fig. 19. A. punctatum, sagittal section near the median plane shortly before the formation of the mouth opening. The section includes a part of the dental ridge in the mandible and the tip of the hyoid arch. Between the two is seen a taste bud forming in the entoderm. The asterisk marks the caudal limit of the ectoderm of the vomerine tooth-germs. Iron hæmatoxylin, fuchsin.

the entoderm and all the yolk granules are drawn. For a short distance in the middle of the figure the nuclei are drawn. A small isolated opening indicates the forming mouth cavity. Below it appear a group of elongated nuclei placed vertical to the floor of the mouth. This grouping of nuclei is the first indication of a taste bud. The outlines of the cells are difficult to see on account of the great number of yolk granules. This taste bud is forming in the entoderm between the tongue and the teeth in the floor of the mouth. It is surrounded on all sides by entoderm crowded with yolk granules and these gran-

ules are closely packed around and among the nuclei of the cells which form the sense organ. It presents the same appearance as do early taste buds in the roof of the pharynx or in any part of the mouth, whether near the ectoderm or far from it.

Another taste bud in a slightly later stage of development is shown in Fig. 20. It is situated in the roof of the mouth just behind the most lateral one of the vomerine teeth. The cells immediately around the tooth together with the three or four deep nuclei at the right hand end of the figure (asterisk) come from the ectoderm of the dental ridge. All the rest of the cells are entoderm. At the left, several nuclei vertically arranged indicate the beginning of a taste bud. It contains

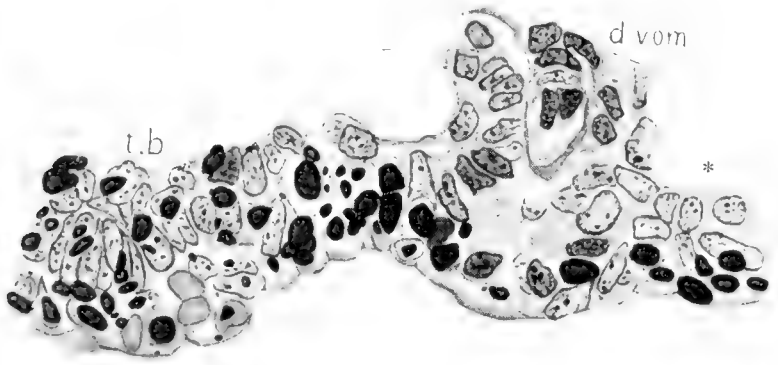

Fig. 20. A. punctatum after the formation of the mouth cleft. Sagittal section of the roof of the mouth in the vomerine region. At the left is a taste bud forming. At the right the asterisk marks the border of the ectoderm. Iron hæmatoxylin, fuchsin.

and is surrounded on all sides by large yolk granules such as are found in this embryo only in entoderm and in parts of the mesoderm. The ectoderm has no yolk at all in embryos of this stage and the nervous system has *very* little.

In Fig. 21 are shown three taste buds and one neuromast from a larva four or five days after the formation of the mouth opening. The entoderm is still clearly distinguishable from the ectoderm and extends beyond the teeth to the lips. At A in the figure is a taste bud standing near the vomerine teeth. The deep nuclei which show active nuclear changes belong to the ectoderm of the vomerine dental ridge. The two cells with dark nuclei belong to the mesenchyme. These cells and the definite outline of the entoderm show that there

is no relation between the taste bud and the ectoderm. At B is a maxillary tooth and a taste bud just behind it. At C is a taste bud which stands just behind the mandibular teeth. Note the yolk granules in both of these, although the yolk is beginning to be absorbed from the entoderm at this time. These are the most anterior taste buds found in Amblystoma and none are found on the

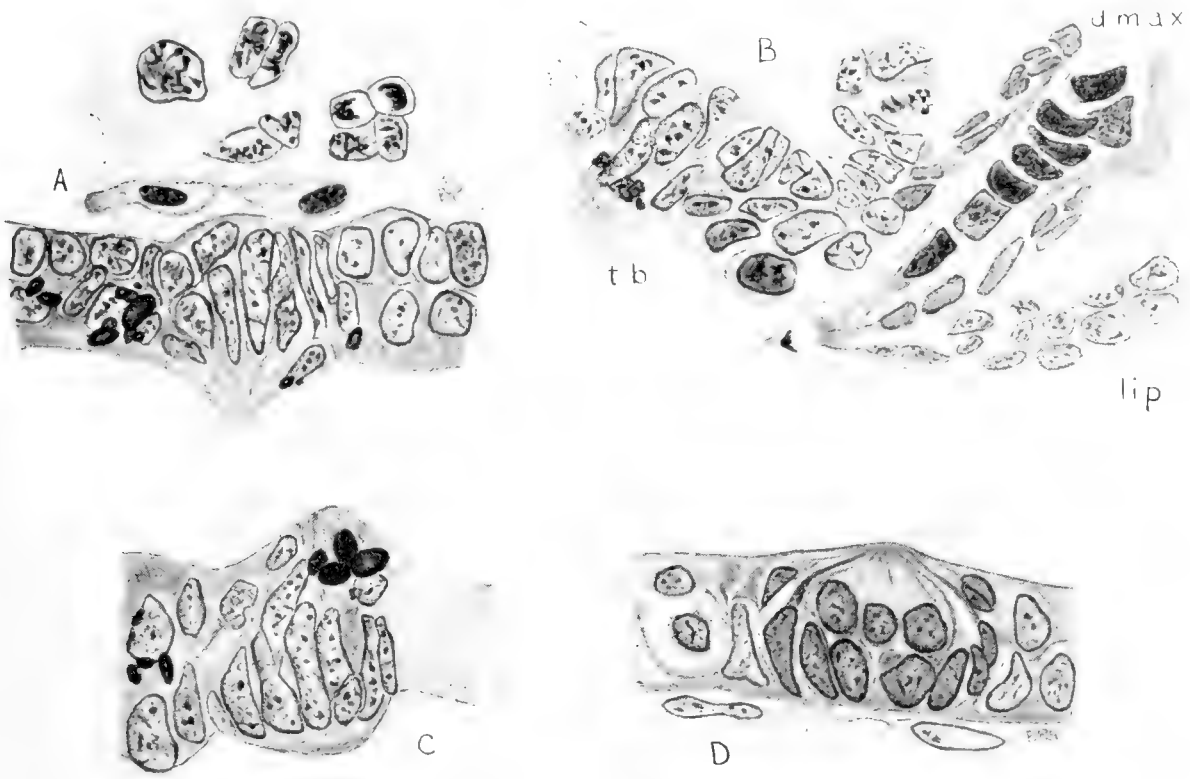

Fig. 21. A. punctatum, buds and neuromast. A, taste bud near the vomerine teeth in a stage later than Fig. 20. B, taste bud just behind the maxillary teeth. C, taste bud behind the mandibular teeth. D, a neuromast from the snout. Iron hæmatoxylin, fuchsin.

outer surface of the head. At D in the figure is shown one of the lateral line organs from the dorsal surface of the snout. Note the pear-shaped sense cells reaching only half the depth of the epidermis.

By the stage represented by this figure taste buds have made their appearance in all regions in which they are found in the adult. Up to this time all the territory in which taste buds appear is lined with entoderm only and the cells of the taste buds themselves are simply re-arranged entoderm cells containing yolk granules. All these points

are demonstrated with the greatest ease. The teeth are formed from ectoderm which migrates in from the borders of the mouth. The tooth germs and the teeth when well formed lie beneath the entodermal lining of the mouth. The teeth pierce the entoderm at about the time that the taste buds are becoming clearly recognizable. Up to this time the entoderm forms a perfectly continuous lining of the whole mouth. It is broken now only by the points of the teeth piercing it. Up to the stage described no ectoderm cells have gained a place in the lining membrane in any part of the mouth. The taste buds are well formed by this stage, their formation from entoderm cells is directly observed in Amblystoma, and they have no relation to ectoderm.

Whether in later stages ectodermal cells of the dental ridges rise to the surface around the teeth and form the covering of the gums or of a larger surface in the mouth I have not yet studied. It may be difficult to determine this point, as the entoderm in the mouth loses its yolk rapidly in the few days following the last stage described. The innervation of the mouth suggests the probability that some part of it is lined by ectoderm. The anterior part of the roof of the mouth, in front of the vomers, receives cutaneous components from the ophthalmicus profundus along with visceral sensory components from the n. palatinus VII (Coghill, 1902). It seems probable that the taste buds are innervated by the fibers of the palatine nerve, as they are always innervated by branches of the communis VII. Whether the trigeminal fibers invade purely entodermal territory or whether ectodermal cells insinuate themselves into the lining of the mouth in this region is unknown. This question, however, has no relation to the question of the origin of taste buds since it is certain that no ectoderm enters into the lining of the mouth until long after the taste buds are formed, if at all. The fact that the trigeminus helps to innervate the mouth, especially in mammals and man, has given tacit support to the idea that the taste buds are of ectodermal origin. The reasoning involved in this assumption is illogical, however, since the taste buds are themselves innervated by the facial nerve which is strictly a nerve related to entodermal surfaces in all vertebrates except those fishes in which taste buds spread into the outer skin.

The presumption, from the standpoint of nerve distribution, is all in favor of the origin of taste buds from entoderm.

Summary of Results.

1. In Amblystoma punctatum the relations of preoral entoderm, premandibular somites, hypophysis and neural tube are essentially the same as in selachians. There are strong indications that the preoral entoderm and hypophysis together constitute the vestige of a palæostoma.

2. The entoderm surrounding the anterior part of the archenteron is compressed and the mouth remains closed until a relatively late period. The location of the palæostoma and of the neostoma can be traced continuously to the time when the mouth opening is formed.

3. The formation of the mouth opening includes three phases: (1) the disappearance of the ectoderm over the mouth area; (2) the relatively long period of solid entoderm; (3) the progressive formation of the oral cavity from behind forward as a cleft in the entoderm.

4. The deep layer of ectoderm gathers into a thickening or collar around the mouth plate and this grows in around the solid entoderm, thus forming the dental ridges. From these are formed the maxillary, mandibular and vomerine teeth. The ectoderm cells and teeth are separated from the mouth cavity by a layer of entoderm. When the teeth are fully formed they pierce this entoderm to enter the mouth.

5. The taste buds are all formed in entoderm. The entoderm lines the whole mouth cavity to the very lips until some days after the taste buds are well formed. The taste buds in all parts of the mouth and pharynx are formed by re-arrangement of yolk-laden entoderm cells. There is no evidence that any taste buds in Amblystoma punctatum are of ectodermal origin. The relations of entoderm and ectoderm are such that it is impossible that any of them should arise from ectoderm in this animal.

ABBREVIATIONS USED IN ALL THE FIGURES.

arch., archenteron.
ch.op., chiasma opticum.
c.s., corpus striatum.
d., dental ridge.
d.max., portion of dental ridge forming maxillary teeth.
d.vom., portion of dental ridge forming vomerine teeth.
en., entoderm.
ep., epiphysis.
h., hyoid arch.
hy., hypophysis.
m., mouth plate.
mand., mandibular arch.
max., upper jaw.
n., neural plate or neural tube.
nch., notochord.
o.ph., oro-pharyngeal cavity.
p., preoral or palæostomal recess in the archenteron.
pr.en., preoral entoderm.
prm., premandibular somite.
r.m., recessus mammillaris.
r.p., recessus præopticus.
r.po., recessus postopticus.
t., terminal ridge or chiasma ridge.
t.b., taste bud.
v.tr., velum transversum.

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THE SKULL OF LABIDOSAURUS.

BY

S. W. WILLISTON,
University of Chicago.

WITH 3 PLATES.

There are not a few problems in the morphology of the reptilian skull which yet await satisfactory solutions. Both paleontology and embryology have failed hitherto to determine indisputably the homologies of the bones of the temporal region, scarcely any two authors being in accord in the use of the terms applied to some of them. Not only is there yet doubt as to the real structure of certain parts of the skull, but the relationships of many groups, dependent as they are so intimately upon cranial homologies, are in dispute. It is evident that the final solution of these problems, problems which resolve themselves at last into questions of relationships, must come from paleontology, and especially from the study of the more primitive and generalized land vertebrates.

Of the paleozoic reptiles the structure of the skull is perhaps best known in the Pelycosauria, thanks chiefly to the studies of Case in recent years. A recent review of the material in this group of reptiles preserved in the University of Chicago museum, among the best now known, that which served Case for the most part in his studies, has convinced me of the general trustworthiness of his results, though, as will be seen, I am not fully satisfied as to the legitimacy of some of his conclusions. That the Pelycosauria are unrelated genetically with the archosaurian reptiles I am now convinced; that they did arise from that branch of reptiles usually called Cotylosauria in the narrow sense, I am also convinced. The resemblances of the skeletal characters between the Cotylosauria and the Pelycosauria have long been evident; the modifications of the pectoral and pelvic girdles in

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either group are of comparatively trivial importance; the remarkable elongation of the vertebral spines in the more specialized pelycosaurs is of scarcely greater moment than are similar modifications among some existing lizards. The presence of the cleithrum, the old-fashioned character of the girdles in general, and the primitive structure of the feet have been recognized in the pelycosaurs, but it has been supposed that radical differences existed in the skull, differences sufficient to separate the group not only ordinally but into a distinct sub-class. My present studies of the skull of *Labidosaurus,* one of the most primitive of the reptiles known from the Permian, prove, satisfactorily to myself at least, not only that there are close morphological resemblances between the skeletons in these two groups of reptiles, but that these resemblances extend to the skull as well; that we have in *Labidosaurus* and its allies, the Pariotichidæ, a persistence of those generalized characters which gave origin to the peculiar specializations of the pelycosaurs; that, in other words, the pelycosaurs are simply an immediate branch from the primitive cotylosaurs, having no direct affinities with the Rhynchocephalia; that their relationships are, probably, far closer with the ichthyosaurs and the proganosaurs. I am aware that McGregor, in his latest studies of the Proganosauria,[1] still adheres to the belief that the group is closely allied to the rhynchocephalian stem, a member not only of the "Diapsida" but also of the "Diaptosauria." His belief necessarily assumes the presence of two temporal vacuities in *Stereosternum* and *Mesosaurus,* which has not been proven and of which I am very skeptical. Moodie recently has ventured the suggestion that the reptiles have arisen exclusively from the Microsauria and not from the Temnospondyli,[2] but, until he finds better arguments to sustain his contention than he has so far furnished, I doubt whether his opinions will obtain acceptance. To evolve five-toed reptiles from four-toed amphibians; to develop a vestigial cleithrum in such forms as *Dimetrodon, Diadectes, Pareiasaurus, Dicynodon,* etc., from forms which did not possess it; to originate a vestigial intercentrum in rep-

[1]Commissao de Estudos das Minas de Carvao de Pedra do Brazil, II, p. 301, 1908.

[2]Geological Magazine, vol. vi, 1909, p. 216.

tiles as an element distinct from the hypocentrum, would seem to demonstrate the fallacy of any arguments in favor of the exclusive origin of reptiles from the known microsaurs. The only legitimate conclusion one may reach is that we have generally erred in the assumption that reptiles arose in Permian times from known types of amphibians. I believe that we have yet to discover the ancestral amphibians, which were neither microsaurs nor temnospondyles in the present sense, at least as far back as early Pennsylvanian times, and I also believe that these ancestors had not only five toes in front, as have the Eryopidæ, with at least the modern reptilian phalangeal formula, but that they also possessed cleithra, and rhachitomous vertebræ.

Of the Cotylosauria the form hitherto best known is, perhaps, *Labidosaurus,* a restoration of which has been published by Broili,[3] with a partial description of the grosser characters of the skull. The material of this genus in the collections of Chicago University, for the most part obtained by the expedition of 1908,[4] is probably the best yet secured. It consists of two excellent skulls, of typical size, three nearly complete skulls of smaller size, and portions of four of five others. Among these smaller skulls is the one described by myself as *Pariotichus* (*Labidosaurus*) *incisivus* Cope.[5] That the specific determination is wrong is very evident. Cope long ago figured the duplicate rows of maxillary teeth in his *Pariotichus incisivus,*[6] whereas this specimen, like the typical labidosaurs, has but a single row, the essential character of the genus, a character indeed which induced Cope to separate the genus from the true Cotylosauria and refer it to the Pareiasauria. That *Labidosaurus* is closely related to *Pariotichus* is, I believe, assured, and its separation by more than family rank therefore improper. That all of these skulls belong in the same species as do the larger ones, *L. hamatus,* is improbable, but their specific determination at the present time is

[3]Paleontographica, li, p. 65, 1904.

[4]Several additional skulls and skeletons were obtained by the expedition of 1909.

[5]Case, Zoological Bulletin, ii, p. 231, 1899; Williston, Journal of Geology, xvi, 139, 1908.

[6]Trans. Amer. Phil. Soc., 1886, p. 290, pl. ii, ff. 4, 5.

of but little moment as having no bearing upon questions of morphological interest.

The two larger skulls, very clearly belonging to the typical species *L. hamatus,* those which have furnished most of the information given in the present paper, are somewhat obliquely compressed, as is often the case with the Permian fossils of Texas; one of them toward the right, the other toward the left. They are of precisely the same size, and the correction of their distortions in the figures is a matter of little difficulty (Plate I and Plate II). The skull, it is seen, is remarkable for its attentuated facial region, and for the beak-like extension of the premaxillaries, terminating in the long, rake-like teeth. The nares, situated nearly at the extremity of the rostrum, are semioval in shape, directed outward. The face in front of the orbits is narrow, gently convex from side to side, with nearly vertical sides and a gentle longitudinal convexity in the middle. The orbits are a little longer than wide, their diameter a trifle greater than the interorbital width. Posteriorly the skull is flattened in the middle above, and greatly expanded in width, the expansion beginning near the back part of the orbits, the lateral margins curving inward at the extreme posterior part. The large pineal foramen is situated near the front part of the parietal bone, about midway between a line drawn through the hind margins of the orbits and the hind margin of the skull in the middle line. There is a pronounced emargination of the hind margin of the skull, extending the width of the parietal bones. In well-preserved specimens the markings of the surface of the skull are very distinct, consisting, for the most part, of round or oval pits forming a reticulation, but not distinctly arranged in rows. In other specimens these pits are less conspicuous, and the surface in some appears almost smooth.

The relations of the bones of the upper surface of the skull have been figured by Case and myself in the papers cited, but I am convinced, from a careful study of the material at my command, that we were both more or less in error in the supposed recognition of elements which we took for granted must have been present. The premaxillæ are separated in several of the specimens in the museum. They unite broadly above with the nasals, by a rounded border in

front of the middle of the nareal margins; and on the sides with the maxillæ, below the nares. The two bones together present a strong anterior convexity, with the alveolar border receding. Each has three, elongated, pointed, slightly recurved teeth, of which the innermost is the largest, the outermost the smallest, less than half the length of the longest. In the closed mouth these teeth, or the inner ones, protrude quite a distance below the mandible, hook-like or rake-like, as shown in Plate II, Fig. 2. This extraordinary development of these teeth and their position, in association with the narrow, compressed facial rostrum, remind one strongly of the phytosaurs and are suggestive of like habits in the living creatures, the exhumation of burrowing invertebrates from the mud or sand of the shores or shallow water. The maxillæ, free in one of our specimens, are rather slender bones, with their greatest width a little in advance of the orbits. They extend back, decreasing in width, to nearly opposite the posterior part of the orbits, uniting above in front with the elongated lachrymals, behind with the anterior prolongation of the jugals. I count in different specimens seventeen teeth, not very different in size, the longest a little in front of the middle of the series, and the series separated from the outermost of the premaxillary teeth by a short diastema. The nasals form the upper side of the rostrum as far as their union with the frontals, a little in advance of the orbits, curving a little downward on the sides back of the nares, whose upper borders only, do they form. The prefrontals are subtriangular in shape and small; their inner sutures begin a little beyond the middle of the upper orbital margin and are parallel with each other, extending a little beyond the end of the frontal bones. The lachrymals are large bones, united broadly with the nasals anterior to the prefrontals, and with more than half the length of the maxillæ below. They form the posterior boundary of the nares and the larger part of the anterior border of the orbits. The precise boundary between the nasals and lachrymals may be somewhat indefinite; the sutural line given is that in which four skulls seem to agree. The frontal bones have nearly parallel sides, extending posteriorly a little beyond the hind margins of the orbits, joining the parietals in a transverse serrate suture, which appears on the under side somewhat in advance of

the line above. The frontals form but a small part of the upper orbital margin. The postfrontals are also small, forming the posterior upper margin of the orbit, and leaving but a small space of frontal margin between them and the prefrontals. The postorbitals are larger than the postfrontals, and also extend a little further back of the frontal suture. They form most of the hind border of the orbits, articulating with the squamosal behind and the jugal below. The jugals begin a little in front of the middle of the orbit in an acute point between the lachrymals and the maxilla. They are broader just behind the orbit, where they articulate with the postorbitals above and the squamosal behind. Below the latter they extend as a rather narrow prolongation to or nearly to the hind angle of the skull, and to the outer extremity of the "epiotic" bones. In the skull figured by Case and myself these posterior prolongations appear to be suturally separated from the broader part of the jugals in advance. A careful examination of other specimens, however, reveals no suture here and leads me to the belief that the supposed suture is merely a fracture in the same place on each side, due doubtless to the fact of the subangular narrowing of the jugal at this place. If there be a distinct bone here I suppose that it must be the real quadratojugal, notwithstanding it has no articulation with the quadrate. All the sutures I have so far described, save perhaps that between the nasal and lachrymal, and that between the postorbital and jugal, are decisively and clearly indicated in the different specimens, some of them conspicuously so, and they, moreover, agree in the different specimens, as long and patient examinations and careful measurements testify. Cope's determinations of the cranial elements in *Pariotichus* and both Case's and my own in the small skull of *Labidosaurus* recognize another suture dividing the so-called squamosal into two distinct elements, though we do not agree in the position of this suture. In the *Labidosaurus* skull figured by myself there does appear to be a divisional line, indistinctly shown and agreeing on the two sides pretty well. Unfortunately in a half dozen other specimens showing this part of the cranial wall, some of them in the most perfect condition both above and below, I can find no trace of a divisional suture, even under the most careful

examination with a lens. I am satisfied that there is none, that there is but a single bone here and not two, and this conclusion was reached before I perceived its significance in comparison with the skull of *Dimetrodon.* This large, flat and thin, or gently convex bone unites on its inner side with the parietal, on the front side with the postfrontal and postorbital, and on the lower or outer side by a very squamous and loose suture with the posterior prolongation of the jugal. This is precisely the arrangement of these bones in *Dimetrodon,* and I am satisfied that the elements are morphologically identical. The chief difference between *Labidosaurus* and *Dimetrodon* consists in the rather large vacuity of the latter piercing what otherwise would be the squamosal, jugal and postorbital bones. For the present I accept Case's determination of the squamosal element as the prosquamosal, but I feel far less assured of its homology than I did formerly, though I doubt not that it corresponds quite with the element in the ichthyosaurs originally named prosquamosal by Owen.

On the posterior or occipital side there are two cranial roof bones on each side, clearly and positively shown in all our specimens, one bordering the hind margin of the parietal, the other the squamosal, and called by Cope respectively the supraoccipital and the tabulare, that is the so-called epiotic of authors. They differ from the bones of the upper surface of the skull in lacking the superficial markings or pittings, and are suturally united with the superior bones at an angle of nearly ninety degrees. The superior or inner of these two pairs of bones, those bordering the parietals, the supraoccipitals of Cope, are the narrower of the two. Their inner ends are curved downward slightly, with an angular interval between them into which fitted the small spine of the real supraoccipital described further on. It has long been believed that the so-called supraoccipital of the Stegocephala and of those reptiles in which a like bone is believed to occur does not correspond to the true supraoccipital of the higher reptiles and mammals. They are clearly membrane bones, and have been called the postparietals by Broom. That they are not the real supraoccipitals is very evident in this specimen in which a large and well defined supraoccipital is found quite dissociated from the

membrane bones of the cranial wall. These bones unite at their outer end with the upper part of the so-called epiotic; the lower, thin and somewhat concave border, is free. The epiotics are broader and longer than this "postparietal," with nearly parallel sides, the lower margin thinned and free and concave in outline, the upper uniting by suture with the squamosal at the angle of the skull. The inner end, which is truncate, unites above with the "postparietal;" below it presents an oblique articular facet for union with the extremity of the paroccipital. The outer extremity is rounded below, and extends to the angle of the skull, articulating apparently with the posterior end of the jugal. On its inner surface near the roof it articulates for a large part of its extent with the hind border of the quadrate. Further observations on the homologies of this remarkable bone will be given later.

The more complete of the two larger skulls has the palatal and basicranial regions in excellent preservation, and but little distorted. Just back of the transverse bones a recent fracture through the narrowest parts of the free pterygoids has permitted the removal of the posterior portion and its complete separation from the encrusting matrix, both above and below, enabling one for almost the first time to obtain a clear conception of the cranial bones and their relations to each other. Very remarkable is the fact that all this portion has no sutural connection with the cranial walls, the suture between the quadrate and the epiotic being the only one, indirectly connecting the vertebral elements with the superior membrane bones. This will readily account for the fact, so often observed, of the loss of the basioccipital and basisphenoid from the remainder of the skull, a loss which, erroneously interpreted, induced Cope to give the name Cotylosauria to the whole group. Above, in the middle, the "postparietals" merely touch the supraoccipital while the epipterygoids further in advance touch the parietals in a mere rounded point.

The quadrate bone of the left side in this specimen lacks its articular head, which had been, unfortunately, broken off with a part of the articular and lost before the discovery of the specimen. The remainder of the quadrate, however, is quite in position, overlapping the pterygoids, and is complete. On the right side the quadrate,

nearly complete, has been entirely separated from its articular relations. The vomers, anterior part of the pterygoids, the palatines and the transverse bones are all in their normal positions. The nares, situated far in front, probably directly below the external orifices, are concealed by the mandibles, which are closed upon the maxillæ; nor is the suture distinguishing the vomers from the posterior elements distinguishable. The narrow pterygopalatine shelf on each side shows, on the upper side at least, a suture between the palatines and pterygoids for a portion of the distance, though I can make out no suture separating the transverse bones, though such doubtless existed. The transverse bones are stout, forming a strong declivity from the plane of the palatines, and they abut massively against the mandibles at least as far as their middle. In the middle, between the pterygoids, opposite and in front of the transverse bones there is a large ovate interpterygoidal space, in front of which the two pterygoids approach each other closely, though not touching. Possibly in the living skull they actually met in the middle. In front of the basisphenoid the pterygoids curve inward so that they meet in the middle behind, leaving no space for a presphenoid or parasphenoid, which is certainly wanting in this specimen at least, though distinctly present in a smaller skull, and recognized by Broili in this species,. The pterygoids unite firmly with the basisphenoid by this inner sphenoid process. Along the margin of the interpterygoidal opening for nearly its whole extent, there is a row, possibly double in front, of small tubercular teeth; a patch of similar teeth is also present in front of the transverse declivity of the palatines, and yet another patch on the summit of each transverse bone.

The posterior prolongations or quadrate processes of the pterygoids, arising just back of the transverse bones from the base of the stout sphenoid processes, are long, thin, divergent, oblique plates of bone, extending back nearly to the hind margin of the skull, articulating broadly but loosely with the plate of the quadrate as shown by the dotted lines in Plate III, Fig. 1. The inner border nearly touches the sides of the basisphenoid; the lower, thin and nearly straight border is continued to near the articular extremity of the quadrate. The basisphenoid is narrow in front, gradually widened behind,

grooved in the middle, shallowly in front, more deeply behind, where it is bordered on each side by a descending process which terminates in a free thin margin underhanging a fossa that opens backward. I cannot distinguish with certainty the sutural division between the basisphenoid and basioccipital, though it seems to be wholly back of the lateral processes, since in another skull, in which the basioccipital has been dislodged, the division has been made back of these processes. On either side of the basiphenoid, or the conjoined basiphenoid and basioccipital, an elongate, cylindrical or oval rod is given off, which is directed downward, outward and backward, lying closely under the posterior end of the pterygoid plate, and reaching nearly or quite to the head of the quadrate. This process, clearly the stapes, seems to be suturally united with the basiphenoid, as indicated in Fig. 1, Plate II. The position of the bone in the specimen seems to be quite normal and undistorted, and the bone is nearly complete, though possibly the extreme end has been broken away; it seems to be perforated proximally by a small foramen. The shape, form and relations of the basisphenoid, stapes and pterygoids may be compared with the author's figure of the same parts in the remarkable rhachitomous amphibian recently described by myself.[7] The basioccipital bone, limited as I believe it to be in front, is small and is clearly distinguishable from the exoccipitals. Its condyle is convex, oval from side to side, somewhat pitted in its middle, and seems to be wholly composed of the basioccipital. The exoccipitals are small, apparently taking no part in the condyle. The suture limiting them from the basioccipital is clearly seen at the sides below and joining the margin of the foramen immediately at the side of the condylar surface above. The suture separating it from the paroccipital passes through the jugular foramen, thence directly upward and forward. The exoccipitals join the supraoccipital by a transverse suture a little below the summit of the foramen magnum. The foramen magnum is heart-shaped, about eight millimeters in its greater diameter. The paroccipitals or opisthotics are distinct elements, the distinguishing suture very clearly indicated, as already stated. They are stout at their base, and are turned outward and backward to end

[7] *Trematops milleri*, Journal of Geology, vol. xvii, p. 636, 1909.

in a short clyindrical rod lying under the proximal posterior end of the quadrate and articulating at the extremity with the facet already described on the lower part of the inner end of the "epiotic." This articular arrangement is the normal one of the opisthotic with the epiotic in the Stegocephala. Chiefly because of this fact I am loath to identify the bone with the quadratojugal, to say nothing of the anomalous position of the bone for a quadratojugal. Anteriorly the suture separating the paroccipital from the supraoccipital passes nearly directly forward to the outer side of the posterior lateral projections of the supraoccipitals where the dividing suture turns inward. Of the suture separating the proötics I am less certain, though it seems to be quite apparent in the position I have figured it in the drawing. The supraoccipital is a large element, when seen from above having a marvelous resemblance to the arch of a dorsal vertebra. A small dorsal spine in the middle posteriorly is intercalated in the angle between the inner ends of the postparietals, but there is no sutural attachment. Anteriorly the two sides of the supraoccipital diverge in the form of zygapophyses, with an emargination between them exposing the cerebral cavity. From the median posterior spine a ridge runs outwards to each "zygapophysis." In front of each lateral projection, the proötic, distinguished suturally, descends in a rounded margin to form the optic notch. In front of these optic notches there is, on either side, a thin, vertical plate, attached either to the proötic or basisphenoid below the meeting in the middle above, leaving an opening of rather small size between them. The upper end of these plates is fractured, but it is very evident that, in position, relations, and shape they agree quite with similar bones bounding the cerebral cavity in most lizards, a small bone, usually lost in the macerated skull, whose homology is not well understood. Since their position is in front of the optic nerve it would seem to preclude the possibility of their being alisphenoids. These elements in the mosasaurs I have identified as orbitosphenoids (see University of Kansas Geological Survey, Vol. IV, Pl. xxix, f. 5; Kans. Univ. Quarterly, Vol. XI, p. 249), but neither identification is quite satisfactory. The upper oblique surface of the pterygoid wings is concealed posteriorly by the quadrates. In front of the quadrates,

and occupying nearly the whole extent of their margin and the upper half of their externo-superior surface, are the elongated and oval epipterygoids. They continue the aclivity of the pterygoid wings on the outer side a little more steeply, ending in an obtuse point a little back of the orbitosphenoid plates, which touches, but is not suturally united with, the parietals above. These epipterygoids are broader in front, where they come in contact with each other over the pterygoids. The quadrates in the larger specimens, and also in one of the smaller, are preserved nearly or quite intact, and in their natural relations. They unite with four bones only, the pterygoids by a very broad and loose union, as shown in Fig. 1, Plate III; with the outer ends of the paroccipitals, as also shown by the dotted lines in the same figure and in Plate II, Fig. 1, and by their posterior everted articular margin with the outer extremity of the postparietals and, much more extensively, with the "epiotics" near the cranial wall. The thin, expanded proximal plate of the quadrate, as shown in Fig. 1, Plate III, narrows into a distinct neck, chiefly by a groove which winds from the under side about the hind margin a little above the articular extremity. The notch thus formed is clearly the auditory notch, corresponding to the notch or foramen in the quadrate of the mosasaurs and lizards; and possibly also it corresponds with the so-called quadrate foramen of the Pelycosauria. Doubtless the Cotylosauria had a small external ear situated nearly as it is in the lizards, above the angle of the mandible. The articular surface of the quadrate for the mandible is oblique to the plane of the bone, so as to look more nearly downward in the normal position of the quadrate. Its outer side projects into a rather narrow process, but does not touch, much less articulate with, the roof bones.

The mandibles, in comparison with the skull, are stout and heavy bones, and amply attest the predaceous habits of the animals. They are slightly expanded in front, where they meet in short symphysis, heaviest and stoutest just back of the orbits and broadest also here. They are nearly straight or gently incurved anteriorly, turned inwards in a broad curve behind. The splenial bones unite in a median symphysis in front and extend back nearly to the articular, leaving a broad, elongate open cavity on the inner side from imme-

diately back of the orbits. They also form a part of the lower margin of the mandible, visible from below as far back as the middle of the orbits, having between them and the hinder end of the dentary, an elongate and acute projection of the angular. The suture between the angular and the articular continues the curve of the inner border of the mandible to the outer side of the extreme posterior end of the angular process. The suture between the angular and the surangular passes forward nearly midway of the mandible, and nearly parallel with the upper border in the closed jaws, to the hind end of the dentary, that is to nearly opposite the posterior end of the orbits. The thin ascending plate of the surangular reaches at the summit nearly as high as the lower margin of the orbits on the inner side of the temporal roof. Over the summit the slender posterior end of the coronoid is visible in the closed jaws, but its anterior part is concealed by the transverse bones. The articular is a short bone, turned inward, with a thin inner margin. It is apparently continued forward as a slender prolongation above the margin of the splenial or angular and forming the lower border of the mandibular cavity, to a slender, acute point nearly as far as the hind end of the mandibular tooth series. Whether or not it is separated from the articular as a distinct bone, the prearticular, or indeed of its precise relations I will not be sure. Sixteen teeth I count in the mandibular series in three different skulls. They resemble the maxillary teeth, but are somewhat smaller. The first or second is distinctly larger than the following ones.

A minute comparison of the skull of *Labidosaurus* with that of *Procolophon* or *Pareiasaurus* would be most interesting and instructive, but since this is impossible for me to make I trust that the figures and descriptions herewith given may furnish the basis for such a comparison by others. With the exception of the form described by Broili (*Seymouria*), I believe that we have no positive assurance of the simultaneous possession of all four roof-bones, supraoccipital, epiotic, squamosal and prosquamosal in any cotylosaurian, using the term in its broadest sense. That all these four elements should occur concurrently in the primitive reptiles is of course to be expected, and Broili's genus seems to furnish an example.

Two of these bones have been lost in all modern reptiles, and, with the exception of the Squamata, three of them. We have generally assumed that the supraoccipital and epiotic of the stegocephs are these two, but I question very much, in the light of the evidence furnished by *Labidosaurus,* whether this assumption is justified. I have long believed that the small element intercalated between the paroccipital and the so-called squamosal of the lizards corresponds to the epiotics of the stegocephs, and I am further confirmed in this opinion by the position and relations of the bones in *Labidosaurus.* However, my views as to the homologies of the cranial arches have been not a little shaken by the structure of the labidosaurian and pelycosaurian skulls, and I shall not venture here to offer any very decided opinions, preferring to wait until further evidence is available, especially that to be yet furnished by the skull of the *Diadectidæ.*

In the remarkable genus described by Cope and Case as *Edaphosaurus* a pair of bones almost certainly homologous with those here provisionally called the "postparietals" are identified by Case as the epiotics, but with a query.[8] In the only known specimen of the genus, the type, these bones and the parietals were crushed down over the supraoccipital nearly to the foramen magnum, and this, I believe, was their normal position. In the restoration given by Case, however, they have been elevated nearly to the top of the supraoccipital, depressing the paroccipitals so far that their outer extremity ends near the articular end of the quadrate. In all probability it articulated, as in *Labidosaurus,* with the outer end of the so-called epiotics, or the inner end of the "quadrato-jugals," as in *Labidosaurus.* The relations of all these bones, the "epiotics," "quadrato-jugals," quadrate and paroccipitals appear to be quite the same in *Dimetrodon, Edaphosaurus,* and *Labidosaurus,* and I doubt not that they are all homologically identical. In *Edaphosaurus,* however, another bone is recognized between the parietal and prosquamosal, clearly corresponding with the additional element supposed to occur in *Labidosaurus,* but which I cannot find. Should it be a distinct element in either or both these genera it does not in the least cast

[8]Pelycosauria, p. 151.

doubt upon the homologies of the two pairs of bones on the posterior border, the squamosal and quadrato-jugal of *Dimetrodon,* the epiotics and quadrato-jugal of *Edaphosaurus,* the postparietals and epiotics of *Labidosaurus. Edaphosaurus* has a temporal vacuity situated much higher than in *Dimetrodon,* by the sides of the parietal bones. Case believes that there is also another vacuity below, separated by a bar from the upper one. No certain evidence of such a vacuity is yet forthcoming, and I shall doubt its presence until demonstrated. I cannot believe that the presence of vacuities in the temporal region of these early reptiles is of the great taxonomic importance that has been ascribed to them.

Perhaps nothing is more noticeable in the skull of the present reptile than the small comparative size of the brain cavity. While the skull measures over seven inches in length and five in width, the foramen magnum is of almost precisely the same size and shape as that of a small *Amblyrhynchus* lizard whose skull measures but sixty millimeters in length. Not only is the foramen of the same size, but the brain cavity also is only a trifle larger in *Labidosaurus.* Small brain capacity is of course to be expected in this old reptile, and, moreover, the size of the brain cavity as compared with that of the skull, may not be a fair criterion of the relative intelligence of the two animals. Nevertheless, that their intelligence was relatively much lower than that of the existing lizards cannot be doubted.

As to the habits of *Labidosaurus* and its allies, the pariotichids, one can hardly doubt that the most of them at least were shore dwellers. The form described by me as *Pariotichus laticeps,*[9] is essentially identical in skeletal and skull structure with *Labidosaurus,* so far as can be determined. Indeed it is not impossible that the form may be a real *Labidosaurus* of a small species. Many of the species of *Pariotichus*, like *Labidosaurus hamatus,* had the peculiarly elongated premaxillary teeth, though perhaps never so exaggerated as in the present species. The only use that I can conceive for such teeth was the seizure of small creatures from burrows, holes or crevices, or for detaching such as may have been closely adherent to rocks. The maxillary and mandibular teeth were not at all pointed, but were, rather more sectorial than prehensile.

[9]Biological Bulletin, vol. xvii, p. 241, 1909.

It is generally assumed that the primitive vertebra from which those of higher types have been developed was composed of several distinct and separated bones, the neurocentra, pleurocentra, and hypocentra, and perhaps others. It must also be assumed that the differentiation of the three or four anterior vertebræ to form the brain capsule took place before the elimination of those parts now absent in the higher animals. We should then expect to find in the vertebrate skull the separate elements or some of them at least, more or less differentiated in the embryonic condition. Furthermore we know that, originally, the ribs were continued as vertebral appendages as far forward as the skull itself, as is evidenced by the atlantal ribs still existent in the crocodiles, pelycosaurs, and other reptiles. And why may we not also assume that the ribs originally continued on the cranial vertebræ? The brain capsule of *Labidosaurus* yet presents so much of its original vertebral condition that it has not become attached to the membrane bones of the cranial walls, and its occipital segment seems to present all the features of an undifferentiated vertebra, the basioccipital representing the hypocentrum, the exoccipitals the pleurocentra, the supraoccipital the neurocentra, while the paroccipitals have all the characters of real ribs. To carry the homologies to the next vertebra we would find the basisphenoid homologous with the hypocentrum, the proötics with the pleurocentra, the alisphenoids or epipterygoids with the neurocentra, while the stapes occupies the ideal position of the primitive rib. That the stapes of the reptiles is not identical with that of the mammals is, I believe, usually conceded; that it did primarily originate as the rib seems not at all improbable. In this connection it is worth while to observe that the stapes of the primitive reptiles seem always of large size, a character also found in both the ichthyosaurs and plesiosaurs.

EXPLANATION OF PLATES.

Plate I.

Labidosaurus hamatus Cope. Skull, from above; two-thirds natural size.

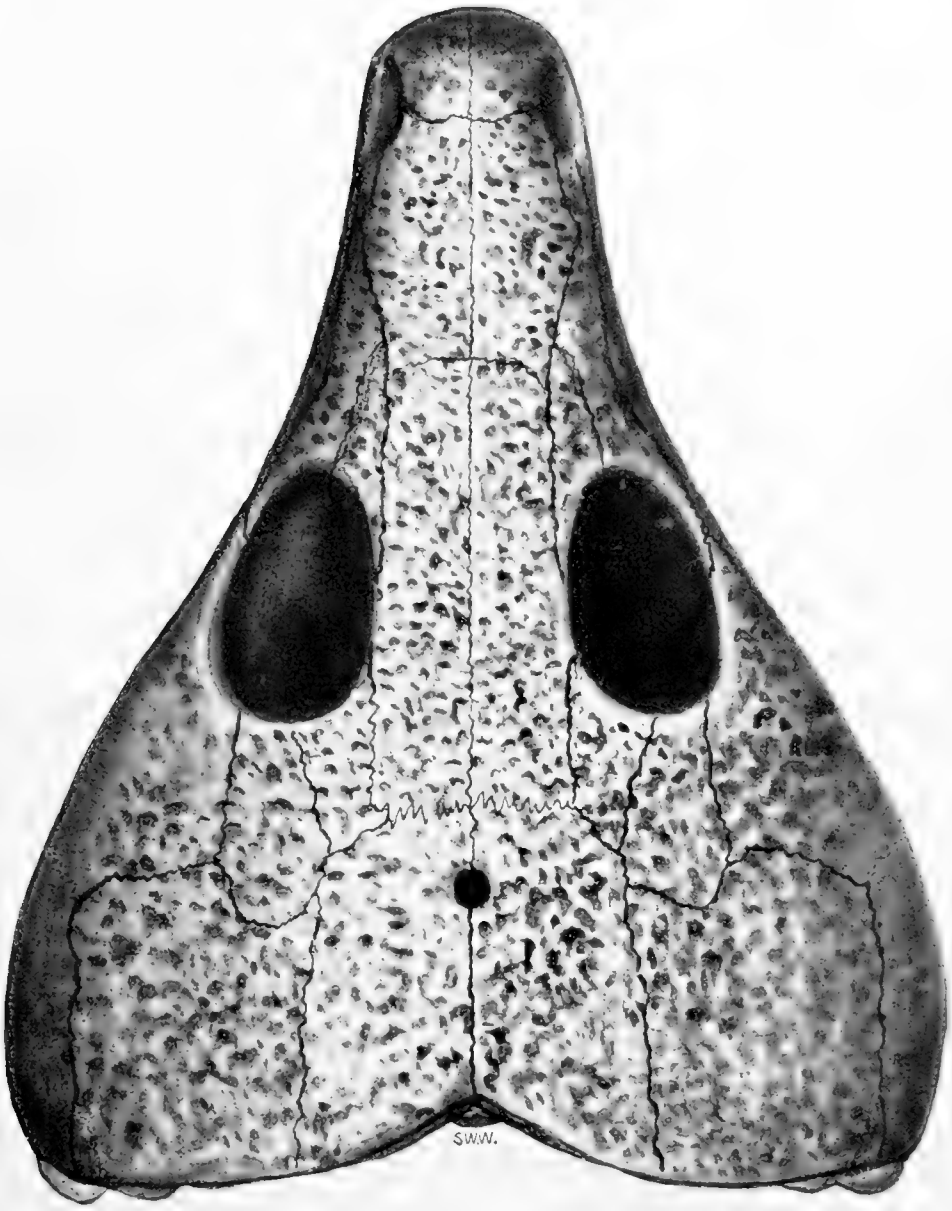
Plate II.

Labidosaurus hamatus Cope. Fig. 1, skull, from below; two-thirds natural size. A, articular; AN, angular; BS, basisphenoid; EP, epiotic; EX, exoccipital; PP, postparietal; PT, pterygoid; Q, quadrate; ST, stapes.

Labidosaurus hamatus Cope. Fig. 2, skull, from the side; two-thirds natural size.

Plate III.

Labidosaurus hamatus Cope. Fig. 1, right quadrate, from below; Fig. 2, the same, from above; Fig. 3, posterior basicranial bones from above; Fig. 4, skull, from behind. All two-thirds natural size. EP, epiotic; EPT, epipterygoid; OC, occipital condyle; PO, paroccipital; PP, postparietal; PR, proötic; PT, pterygoid; Q, quadrate; SO, supraoccipital.


S.W.W.

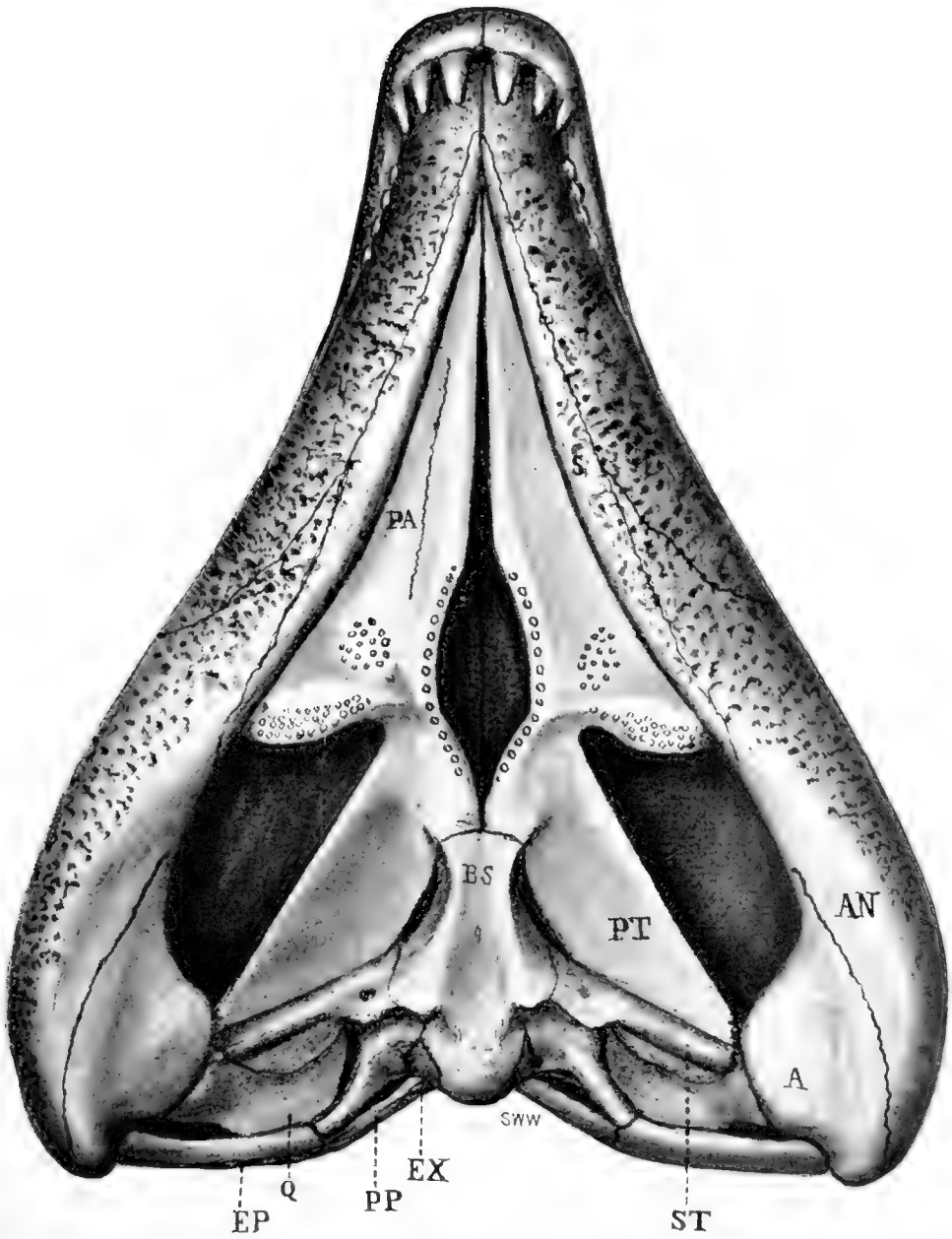


FIG. 1.

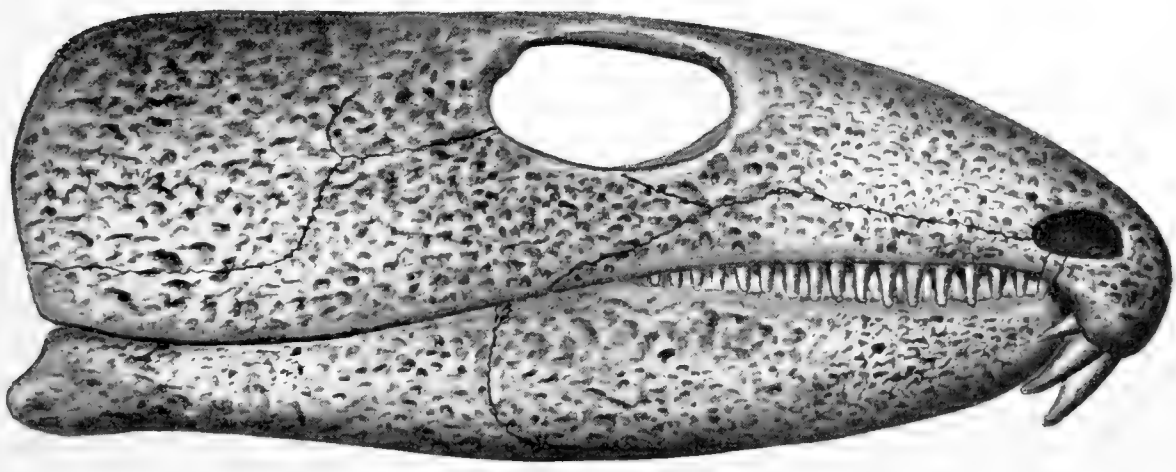
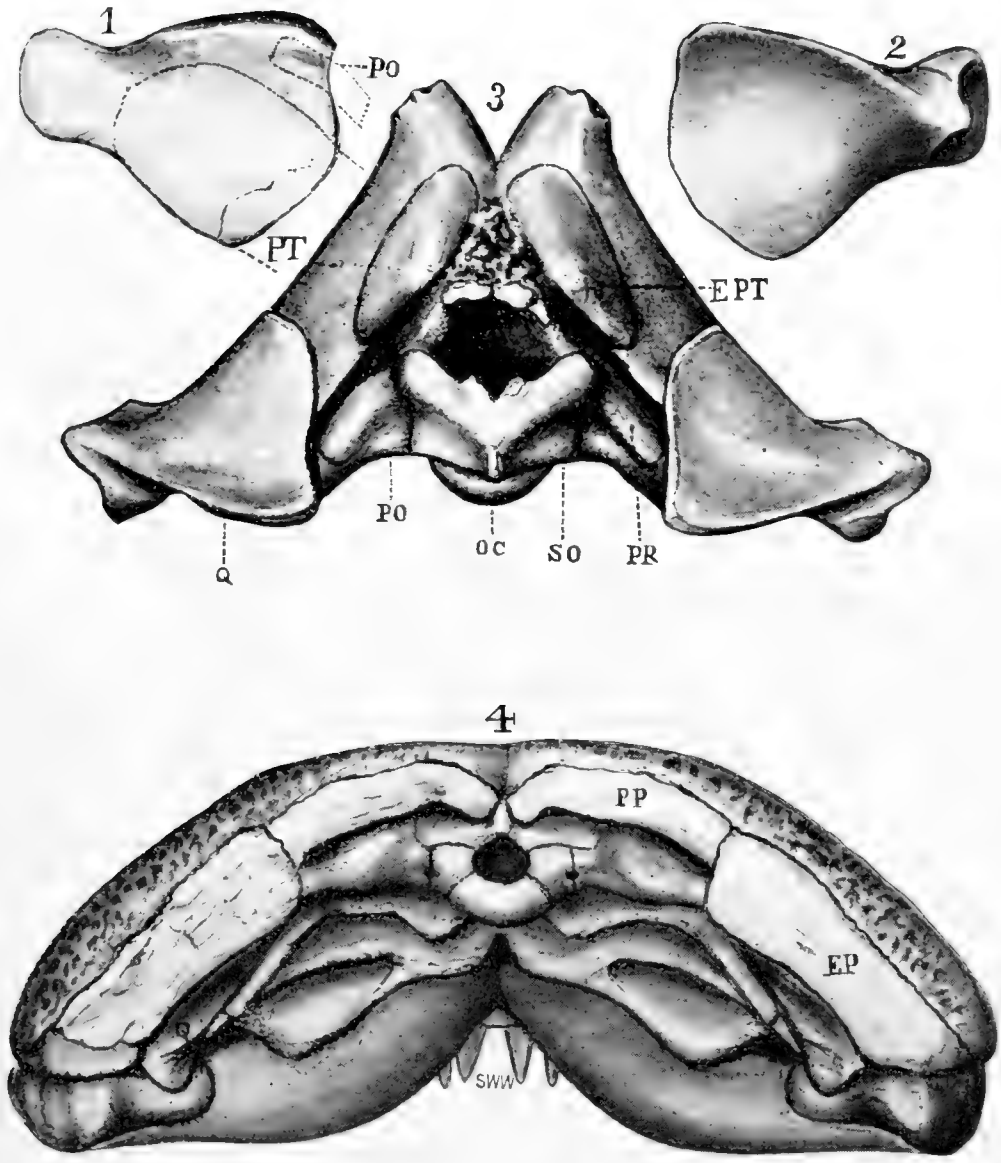


FIG. 2.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A HUMAN EMBRYO WITH SEVEN PAIRS OF SOMITES MEASURING ABOUT 2 MM. IN LENGTH.

BY

WALTER E. DANDY.

From the Anatomical Laboratory, Johns Hopkins University.

WITH 6 PLATES.

Among the youngest human embryos and one of the youngest in Professor Mall's collection, is Embryo No. 391, which he has very kindly permitted me to reconstruct and describe. In general development this embryo is almost identical with, probably a trifle older than, the Kroemer-Pfannestiel embryo, Klb, which measures 1.8 mm. and has six pairs of somites. It is older than Eternod's embryo measuring 1.3 mm., Graf Spee's Gle 1.54 mm., and Embryo Frassi 1.17 mm., in the order named. It is younger than the embryos of Unger and Bulle, of 9 and 14 somites respectively, Eternod's 2.1 mm. embryo, and embryos XLIV (Bff), LXVIII (Lg. 2.15 mm.), VI (B. R. 2.2 mm.) and VII (E 2.1 mm.) of His.

This very rare specimen came into Professor Mall's possession through the kindness of Dr. R. W. Pearce, of Albany, New York, with the following history from the physician who handed the specimen to him. "The woman had passed her period about two weeks when she performed an abortion with a stick about 8 inches long, which she whittled out for the purpose. This she passed into the uterus and 24 hours later this specimen was aborted. Her purpose in calling me was to see if her object had been attained. I have kept the specimen two years in a bottle of weak formaldehyde."

Upon receipt the following measurements were made by Professor Mall; ovum 16 x 14 x 12 mm., embryo about 2 mm. The specimen was placed in fresh formalin, stained with alum-cochineal-eosin, imbedded in paraffin and cut into serial sections 10 microns in thickness.

From these sections (163 in number, excluding the bauchstiel), the embryo was reconstructed upon a scale of 200 magnification,

giving the model a total length of 32.6 cm. A shrinkage space between the layers of tissue aided materially in the reconstruction without sacrificing tissue. The model is so constructed that every detail may be exposed by cuts and windows.

Upon presentation of an embryo the question naturally arises, is it normal? The history of a mechanical abortion points very strongly toward a normal embryo. It compares very closely with the few other known embryos of this period. The tissues, including the chorion, are excellently preserved and show no degenerative changes.

Age.

From the history that the menstrual period passed about two weeks, the age of 13-14 days would fit very nicely into the conventional Reichert-His theory of fertilization and age of embryos. Recent observations however by Mall in 1000 cases with menstrual history have shown that we underestimate the age of young embryos, on an average about 10 days, that fertilization is not restricted to the period immediately before the menstrual period, but may occur at any time in the intermenstrual period. Bryce and Teacher also came to the same conclusions and illustrate this in a well constructed diagram of the menstrual cycle. This obviates the remarkable distortion which has been necessary to harmonize age with size and progress of development, and makes the human embryo much less precocious in its development. In conformity to the above observations the true age of this embryo is probably about 24 days, which would make the time of fertilization occur about ten days before the time for onset of lapsed menstrual period or about eighteen days after beginning of the last menstrual period.

The Adnexa.

Chorion. The chorionic membrane is about 0.1 mm. thick, and is covered with many branching villi varying in size up to 1.25 mm. in length and 0.1 mm. in thickness. These villi are more numerous at the point of attachment of the bauchstiel and gradually fade away on all sides until finally a clear zone results from their absence on the opposite pole.

The chorionic membrane and villi have the characteristic inner loose mesenchymatous layer of beautiful branching spindle and stellate cells with anastomosing processes and a somewhat jelly-like intercellular substance; an external double epithelial layer, consisting of the inner Langhans layer of small cells with lightly staining nuclei and cytoplasm, and the outer syncytial layer which stains more deeply with eosin and has larger and more densely staining nuclei, but no definite cell boundaries. From the epithelial layer of the chorionic membrane and villi, numerous buds develop, some from the syncytial layer alone, others from both layers of epithelium. These represent proliferation and new formation of villi. The mesenchymatous layer of the chorionic membrane contains many newly forming capillaries, some of which extend into the villi. The details of these will be considered later in the description of the vascular system.

The Bauchstiel. The bauchstiel does not differ from that of other young embryos. It consists of loose mesodermal tissue, lined externally by a single layer of flattened mesodermal cells. It is continuous distally with the chorionic mesoderm and proximally with the mesoderm of the umbilical vesicle, amnion and body of the embryo. It contains the allantois, umbilical arteries and veins and their resulting sinuses and branches.

Umbilical vesicle.—The yolk sac has no stalk but is attached to the entire length and breadth of the embryo, with the exception of small portions of the anterior and posterior ends, which are covered by the short head and tail folds of amnion. At its attachment to the embryo, the walls are very thin, consisting of two layers of flattened cells—mesoderm and entoderm. These walls gradually grow thicker distally, due to the degree of development of the blood islands, which also cause a great distortion and knotty appearance of the mesodermal surface. This vascular development extends throughout the whole length of the umbilical vesicle, but is practically limited to the ventral or distal half, only a few islands being seen in the dorsal half. The greatest development seems to be near the center of the vesicle and is apparently developing to connect with the future vitelline veins. This is in contradistinction to the findings in Eternod's 1.3 mm. embryo, in which the posterior region

of the umbilical vesicle is drained by the sinus ensiforme. The entodermal lining of the inner surface of the umbilical vesicle forms a distinct layer in places, whereas in other locations, especially in the ventral half, no definite layer of cells can be made out, so intimately is it fused with the knotty and thickened mesoderm.

Amnion.—The amnion is a completely closed cavity, with very thin delicate walls of flattened cells, the individuality of ectoderm and mesoderm being everywhere beautifully preserved. The cavity of the amnion is very large, probably due, as suggested by Professor Mall, to the osmosis of large quantities of dilute formalin in which it was preserved for over two years. Anteriorly a rather deep pocket of amnion dips ventral to the heart, evidently a sign of posterior extension of the head fold. The tail fold is very small, covering the embryo for a distance of only 5 or 6 sections.

General Appearance of the Embryo.

The embryo presents an anterior and posterior elevation with a marked dorsal link. (Plate IV.) The anterior elevation is gradual, the posterior very sharp, rising at an angle of about 80°. This kink seems to be partly natural and partly an exaggerated post-mortem condition. We should naturally expect a dorsal concavity due to the greater development of the structures in both the anterior and posterior regions of the embryo. The marked accentuation however may be due, as suggested by Professor Mall, to the large amnion filled with fluid, the weight of which would naturally act upon the point of least resistance. Shrinkage incident to manipulation and imbedding also plays an important rôle in its aggravation, especially after location of the point of least resistance. This is clearly shown by a comparison of the model with a sketch of the embryo before imbedding, there being an accentuation of the kink by almost 15°.

Ectoderm.

Nervous system. The extent of development of the nervous system is a medullary groove, open throughout its entire length. The brain region is divided into three primary vesicles (Plate VI), the anterior being equal in size to the other two combined. The first or

anterior vesicle is long, wide and deep with edges everted and projecting outward over the anterior and lateral walls of the anterior body elevation. The walls of the second and third vesicles have a tendency toward inversion and the enclosed vesicles are more sharply defined laterally than the first. The brain passes insensibly into the spinal cord, which is much smaller in all diameters, but is nowhere closed. Posteriorly the medullary groove forms a shallow dilatation gradually fading into the flattened surface of the primitive streak. (Plate IV.)

No neurenteric canal is visible, though present in the younger embryos of Frassi, Graf Spee and Eternod and possibly the Kroemer-Pfannenstiel Klb. No traces of spinal or cerebral ganglia or nerves are visible. No suggestion of anlage of the lens, optic or otic vesicles could be detected.

Primitive streak. Posteriorly and dorsally in the region of the termination of the notochord and neural groove, ectoderm and mesoderm gradually losing their individual morphological characteristics, fuse to form a mass of a single variety of simple undifferentiated cells, which extends to the posterior termination of the embryo. This is the primitive streak (Fig. 7). The entoderm also seems to be a part of this mass since its cells are directly continuous with it, although the characteristic lining of the hindgut is still maintained throughout. There is no groove except a very shallow flattened dorsal depression which is gradually lost posteriorly and which is continuous anteriorly with the neural groove. This primitive streak region suggests a storehouse of simple undifferentiated cells supplying mesoderm, ectoderm and entoderm in the earlier stages, later becoming differentiated into the characteristic morphology and arrangement of the different layers.

Entoderm.

Entoderm lines the ventral surface of embryo, fore and hind gut, allantois and the inner surface of the umbilical vesicle. In the ventral entoderm of the embryo is a median longitudinal groove corresponding to the location of the notochord and caused by its adherence to the ectoderm of the medullary groove. On either side of this groove and parallel to it is a ridge caused by

ventral projection of the dorsal aortæ. These are no doubt accentuated by post-mortem shrinkage. The opening of the foregut is straddled by two prominent ridges which unite above into a large ventral bulging, represented by the overlying cœlom, and pericardial cavity with the enclosed heart, respectively.

The foregut is present in 32 sections, representing a length of 320 microns. It ends blindly anteriorly and is separated by mesoderm from the buccal cavity which is forming by an invagination of ectoderm. There is however no bucco-pharyngeal membrane. One small pharyngeal pouch is present on each side below the first and only complete aortic arch. These pouches are continuous ventrally and unite to form a ventral pouch. There is no contact with the outer ectoderm, although the mesoderm separating them is considerably thinner than elsewhere.

The hindgut is a blind somewhat oval, dilated pouch, 120 microns in length by sections, but on account of the dorsal kink of the embryo and the consequent partially longitudinal plane of the sections, the actual length is somewhat greater. The allantois arises from the ventral surface of the hindgut. It passes from the embryo into the bauchstiel in company with and between the umbilical arteries. After the union of these arteries, it takes its position between the arterial and venous sinuses (Plate VI) and bends at almost a right angle to conform to the direction of these sinuses and the bauchstiel. At this latter point it divides into a short stub measuring about 40 microns and a much longer branch. The terminus of each branch may be seen in Figs. 8 and 9. The allantois is lined by a single layer of cubical epithelium; it maintains a lumen to the point of division, from which it consists of a solid core of cells.

No liver or thyroid anlage has made its appearance. No cloacal or bucco-pharyngeal membranes can be distinguished.

Notochord. With the exception of the very posterior tip which is entirely free for a distance of three sections, the notochord is everywhere fused with and apparently an integral part of the entoderm. Posteriorly it is very conspicuous as a relatively large and very compact knob of deeply staining cells on the dorsal wall of the hindgut. This sharply differentiated mass invaginates into the ectoderm, with which is it not here united (Fig. 6). It gradually

becomes smaller anteriorly, being represented by a very small thickening of entoderm (Fig. 3) and finally is indistiguishable. Except in the region of hindgut mentioned above, it, together with the entoderm, is everywhere in contact with the neural groove ectoderm, usually a line of contact being visible; in some places however a line of demarcation is exceedingly difficult to make out. No trace of a notochordal canal could be detected.

Mesoderm.

Mesoderm.—Posterior to the somites is a large mass of very simple undifferentiated mesoderm which dorsally passes insensibly into the primitive streak. It is largely responsible for the size and shape of the posterior region of the embryo. Its only differentiation is laterally, where it becomes somewhat more compactly arranged, lining the spaces which are forming the peritoneal cœlom. Anterior to the somites the paraxial mesoderm is also very simple and serves as a wedge of tissue between entoderm and ectoderm. The highest mesodermal differentiation is seen in the formation of the somites, cœlom and heart which will now be considered.

There are seven pairs of somites completely segmented. Each of the first six pairs contains a cavity, unconnected laterally with the cœlom as described by Keibel in the reconstruction of the Kroemer-Pfannenstiel embryo, Klb. The first pair is apparently the most highly differentiated, being the largest, having the largest cavity and most compact peripheral arrangement of cells and is connected laterally by the most clearly defined and differentiated intermediate cell mass. These characteristics are less pronounced in each successive posterior somite until the last is the smallest, contains no cavity, has no cellular arrangement, and shows no other signs of differentiation. This would seem to indicate that the cavity is of secondary origin; Graf Spee however finds a small mesodermal slit in embryo Gle which he thinks is a myocœle, although no somites are present. In addition to the completely formed somites, another pair is just beginning to show signs of constriction from the posterior paraxial mesoderm. Anteriorly there is also another pair of somites about half segmented with a very uniform compact mass of cells but containing no cavity. This rudimentary pair probably represents the

first pair of somites, which according to experiments in the chick by Marion Hubbard and Patterson, is established first in time and position, but develops slowly and maintains its connection with the anterior paraxial mesoderm, thus causing the formation of somites to take place only posteriorly.

Cœlom. In his article on the cœlom for the new Keibel-Mall Embryology (now in the press), Professor Mall used this embryo as the second stage of human cœlomic development. Graf Spee's Gle 1.54 mm. is the first human embryo to show any sign of cœlom formation, this being a very small bilateral slit in the mesoderm of the anterior portion of the embryo—the beginning of the pericardial cœlom. This slit is said to communicate externally by a very small channel. The next stage, represented by this embryo, shows a very large and well developed single, united pericardial cavity. (Figs. 1 and 11.)

The posterior continuation of the pericardial cavity on each side (Figs. 11 and 15) probably represents the pleural cœlom, which passes insensibly into the peritoneal cœlom, now forming from multiple foci. A glance at Fig. 11 will show the extreme irregularity and entire absence of any metameric arrangement in the formation of the peritoneal cœlom. Numerous irregular pockets of varying size dip into the mesoderm from the extracœlom. This communication with the exterior may be either primary or secondary. The diagram of the reconstructed cœlom, however, would seem to indicate the former might be the case, because posteriorly a rather long continuous slit is present in the mesoderm, the pockets of which are shorter, much wider at the external opening, and diminish in size internally. There are, however, several small independent cavities, having no visible connection with each other or with the extracœlom, which strongly suggest an independent formation by internal mesodermal cleavage, beginning in multiple small foci, which may later connect with the other cavities and thus indirectly through them to the extracœlom. Judging from the structure of this specimen alone it seems probable that the result may be a combination of both processes which are in reality ultimately one and the same process differing only in position. This irregular formation has been described by

Bonnet in young sheep embryos, both in pericardial and peritoneal cœlom, the latter only communicating with the extracœlom.

The pericardial cavity communicates freely with the pleural, which in turn passes into the peritoneal cœlom and through it communication is made on each side with the exocœlom at the level of somite IV (Fig. 11). There is therefore a complete circuitous canal, by which it is possible to travel from the extracœlom on one side through the embryonic cœlom to the extracœlom on the other side.

Heart. The mesodermal walls of the heart are the result of confluence, fusion and absorption of the walls of the pericardial cœlom. The heart is attached dorsally throughout its whole length by a mesocardium; ventrally and laterally it is free in the pericardial cavity. The heart roughly fills about one-third of the pericardial cavity, is bent upon itself almost at a right angle, with convexity to right and concavity to the left side of the embryo. The endothelial heart is a simple tube of flattened cells lying within the mesodermal walls but greatly shrunken and collapsed from fixation.

Nephric system. There is no evidence of the Wolffian bodies or ducts. Three independent small cavities are seen in the lateral masses of somites I and II on the right side and somite II on the left side, none of which connect with cœlom. These cavities show a partially differentiated wall of more or less distinct cubical epithelium (Fig. 3) and are probably the earliest stages of the pronephric tubules. Below somite II the lateral masses become very vague and indistinct. Fig. 11 shows a number of cavities, some independent, others connecting with the cœlom, but agreeing in position with the future lateral masses. They are merely small undifferentiated slits in the mesoderm, have no definite lining and may or may not be homologous with those mentioned above as pronephric tubules and in earlier stages of development. They may, however, represent foci or cœlomic development, as is suggested by several small spaces similar in position and connecting with the cœlom.

Vascular system. The description of the vascular system of this embryo might appear very unorthodox indeed, were it not for the previous description by Eternod of a 1.3 mm. human embryo, which for a decade has remained alone and unconfirmed, which shows, how-

ever, all the signs of a normal embryo. The question of a primitive yolk circulation has always been regarded as an established and indisputable fact, based almost entirely upon comparative embryology.

Recent observations in human embryology, however, have shown the human ovum to differ markedly from other comparative forms in the very early stages of development. This embryo, together with the early embryos which have been described, particularly that of Eternod, seems to indicate that the primitive human vascular system differs from the conventional comparative system in that the umbilical circulation is the predecessor of the time-honored vitelline circulation.

In studying the vascular system, every capillary was traced as far as high magnification would permit. The recent work of H. M. Evans, with injections of fresh specimens of young chick and pig embryos, has however demonstrated the existence of large hitherto unrecognizable capillary beds and has shown the comparative inefficiency of studying fixed uninjected tissues for blood-vessels, even under the most favorable conditions. We must therefore always bear in mind the possibility of collapsed vessels, and that other things being equal, too much emphasis cannot be placed upon negative evidence. Even if there should be some small capillary beds, which are unrecognizable by the microscope in the absence of injections, it would merely be evidence in favor of vascular connection between the umbilical vesicle and the embryonic circulation, and would not in any way affect the question of priority, because of the relatively greater development of the umbilical vessels. A glance at Fig. 15 and Fig. 12 will conclusively show that the functioning system is umbilical or chorionic and that if a connection could be traced through the large gap between the umbilical vesicle and the small sprout of the possible vitelline vein, it would be comparatively insignificant. Since the embryonic vessels are full of blood and there is no apparent connection with the blood-forming area in the umbilical vesicle, the question naturally arises, how are we to account for the presence of the blood corpuscles? This is explained by finding many beautiful examples of endothelial proliferation of blood corpuscles from the capillaries in the chorionic membrane (Fig. 10), probably supplying the embryo with blood until the time of connection with the yolk sac.

A survey of the literature gives very limited information on the primitive vascular development. The first suggestion of a vascular anlage is a very indistinct knotty appearance in Peters' embryo; Keibel, however, who made a reconstruction of this ovum, says this appearance is merely suggestive and that no positive conclusions can be drawn. None of the several other young ova show any vascular formation. Embryo Frassi 1.17 mm. shows the first blood vessels, which are distributed in the chorion, bauchstiel and ventral pole of the yolk sac. No vessels were observed in the body of the embryo. Graf Spee's Gle 1.54 mm. is probably next in point of age, followed very closely by Eternod's 1.3 mm. embryo. Graf Spee describes the first anlage of the heart, otherwise there are no vessels in the body of the embryo; blood islands are present in the ventral pole of the yolk sac, but no mention is made of vessels in the bauchstiel or chorion. Eternod's 1.3 mm. embryo is the first specimen to present a complete circulation. It has a very well developed umbilical circulation and the villi are partly vascularized, but no vitelline vessels could be detected. The Kroemer-Pfannenstiel embryo Klb. of approximately the same age as the embryo under consideration is said to have a very large umbilical artery, unaccompanied however by a corresponding vein, also omphalomesenteric veins are present but no corresponding arteries. The vessels in the yolk sac are full of corpuscles, but no mention is made of any chorionic vessels.

This summary of the earliest vascular development is by no means evidence in favor of a primitive vitelline circulation. Although the facts are very meager on account of the scarcity of material and nothing positive can be deduced, nevertheless there seems to be some evidence in support of a primitive umbilical circulation. The first embryo having a complete circulation is Eternod's (1.3 mm.), with a well developed umbilical circulation. From the other remaining specimen (Kroemer-Pfannestiel Klb.) between Eternod's and our embryo it would be hard to draw definite conclusions on account of the presence of the artery of one system and the vein of another, forming no complete circulation.—

The presence of capillaries in the chorion, bauchstiel and yolk sac in Embryo Frassi would seem to indicate that the mesoderm which forms all of the above structures, is endowed with a high power of

vascularity in its early history, irrespective of position, and that from this vascular formation, whether from single or multiple points of origin, two main primitive vascular areas originate practically synchronously and develop independently toward the embryo. Eternod's embryo together with the one under consideration, which are the two earliest embryos with a complete circulation, would seem to indicate that the umbilical circulation was the first to attain this function.

As mentioned before, this embryo agrees in general very closely with Eternod's 1.3 mm. embryo, differing only in a few details. The yolk vascular system is developing rapidly and consists of numerous large blood islands, forming irregular, disconnected channels and masses of blood-forming tissue, situated almost entirely on the ventral half of the umbilical vesicle (Fig. 12), extending from the anterior to the posterior poles and more marked in the region of the future vitelline veins. A few very small blood islands extend along the dorsal half of the yolk sac, but never is there any visible vascular connection within the embryo and always a considerable distance exists between the blood islands and the vessels of the body of the embryo.

The umbilical circulation consists of a simple vascular cycle comprising the umbilical veins, heart, dorsal aortæ and umbilical arteries, with capillary connection in the chorion and chorionic villi. A number of villi show the early stages of vascularization, which are not limited to the region around the attachment of the bauchstiel, but extend anteriorly beyond a point corresponding with the anterior limit of the embryo. There appear to be several seemingly independent small systems of capillaries in the chorionic membrane, into which drain the capillaries of the villi. Although these systems seem to have no connection with each other, the difficulty of following capillaries in uninjected specimens would make a positive statement impossible. As mentioned above, many of these chorionic vessels show beautiful examples of what appears to be blood formation from endothelial proliferations (Fig. 10) and is probably the source of the blood for the present circulation.

Two large independent blood reservoirs (Figs. 8, 9 and 15) in the bauchstiel connect distally with the chorionic vessels; proximally

the smaller gives off the paired umbilical veins; the larger is formed by the confluence of the umbilical arteries.

The umbilical veins run in the dorsolateral portion of the body of the embryo, at the point of origin of the amnion. The left vein gives one, the right vein two, very short branches to the amnion at the level of the posterior region of the embryo. Nothing comparable to the sinus ensiforme of Eternod's 1.3 mm. embryo was observed. While still in the dorsal position and just before its course ventralward, each umbilical vein gives off a short branch which runs ventrally through the body of the embryo toward the umbilical vesicle. On one side it terminates before reaching the yolk sac, on the other side it runs a short distance in the wall of the yolk sac and seems to end blindly. On each side a couple of spaces, presumably capillaries, although they do not contain any blood, are in the immediate vicinity of these short branches, but no connection can be detected. These short branches and possible capillaries may be the beginnings of the vitelline veins, although the dorsal point of origin from the umbilical veins is rather against this view, unless there is a subsequent ventral wandering, as has been observed in many other vessels. This is the only visible evidence of the possibility of the vitelline veins. (Plate IV.)

The umbilical veins now run ventrally around the cœlom and unite to form the heart just anterior to the origin of the foregut. It is a simple tube of endothelium throughout. From its anterior extremity one complete aortic arch is given off on each side (Fig. 1) and in addition, four short stubs on the right and two on the left sides, which represent rudiments of future aortic arches. No corresponding buds are given off by the cephalic portion of the dorsal aortæ to connect with these. The heart is a step in advance of that of the Kroemer-Pfannenstiel embryo, which has one aortic arch on each side, but a paired heart is still present. Eternod's 1.3 mm. has a slightly higher development, in that three and possibly four complete arches are present on each side.

The dorsal aortæ, paired throughout, begin anteriorly in a small dilatation and terminate caudally at the posterior border of the foregut in a much larger dilatation, from which the umbilical arteries are the direct continuation (Plate VI). Each aorta gives off a series of

eleven lateral branches, beginning as very minute and rather indefinite branches posterior to the first somite; these become progressively larger and reach a climax at the posterior dilatation of the aorta. In the somite region they are solid buds (Fig. 5), the last five arising from the dilatation have a lumen, but end blindly in the mesoderm of the splanchnopleure (Fig. 5). On the right side the last three branches seem to end in a common blood island, giving the impression of a small capillary plexus; this was, however, not observed on the other side. These branches probably represent the segmental arteries of Mall destined for the umbilical vesicle. In the somite region there are six of these branches for the seven somites; on one side all six arise between the somites; on the other side three arise intersegmentally, the other three come off nearer the center of the somites. From the dorsal and caudal end of the posterior aortic dilatation there is a very short stub on each side, which may represent the beginning of the caudal aorta. Otherwise no dorsal branches of the aortæ were observed.

The umbilical arteries, somewhat larger than the aortæ, are direct posterior continuations of the posterior aortic dilatation. In the bauchstiel they unite to form a large sinus (Fig. 9) which lies posterior to the smaller venous sinus. (Fig. 8.) It sends off numerous large branches to the chorion, some of which anastomose and redistribute branches to the chorionic membrane and villi.

It is a pleasure to extend my heartiest thanks to Professor Mall for his kindness in allowing me the privilege of describing this very rare specimen, for his valuable advice and suggestions and for the use of two manuscripts which are not yet in print.

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EXPLANATION OF PLATES.

Plate I (Figs. 1-7).

Fig. 1. Section 22, × 50. B1, First primary brain vesicle; Oo, Aorta; A1, Complete view of longitudinal section of first aortic arch; A2, Cross section of stub of second aortic arch; En, Endothelial heart; Ht, Mesodermal wall of heart; P.C., Pericardial cavity; Fg, foregut; Am, Amnion; Mes, Mesoderm; Ent, Entoderm.

Fig. 2. Section 53, × 50. Ch, Chorda; Ao, Dorsal aorta; V, Umbilical vein; Fg, Foregut; P.C., Pericardial cavity; B2, Second primary vesicle of the brain.

Fig. 3. Section 76, × 50. Pr, Pronephros; Coe, Cœlom; Pl, Pleural Cœlom; V, Umbilical vein; Br V, Branch of umbilical vein, only suggestion of a vitelline vein, it unites with umbilical vein two sections below; Ch, Chorda; M1, First Somite in each side.

Fig. 4. Section 93, × 50. Br, Cross section of lateral branch of dorsal aorta; V, Umbilical vein; M4, Somite IV, with cavity (anterior tip of somite IV on opposite side); Coe, Cœlom; E.C., Communication of cœlom with exterior; Ch, Chorda.

Fig. 5. Section 101, × 50. Br. Lateral branch of Aorta; Ao, Aorta; V, Umbilical vein; M4, Somite IV with cavity; M5, Tip of Somite V; Coe, Cœlom.

Fig. 6. Section 137, × 50. Ao, Dorsal aorta; Br, Lateral branch of aorta (branch on left side terminates in a mass of mesodermal cells which may be a small blood island); Ch, Chorda at point of greatest development; Coe, Cœlom; S.M, Slit in mesoderm. (See also Fig. 11.) Pr. S, Beginning of primitive streak. U. V, Umbilical vesicle.

Fig. 7. Section 152, × 50. Shows primitive streak region (Pr. S.) from fusion of ectoderm, mesoderm and entoderm. Hg, Hindgut; All, Allantois, near point of origin from hindgut; U, Umbilical artery; V, Umbilical vein; S. M, Slit in mesoderm. Ect, Ectoderm; Ent, Entoderm; Mes, Mesoderm.

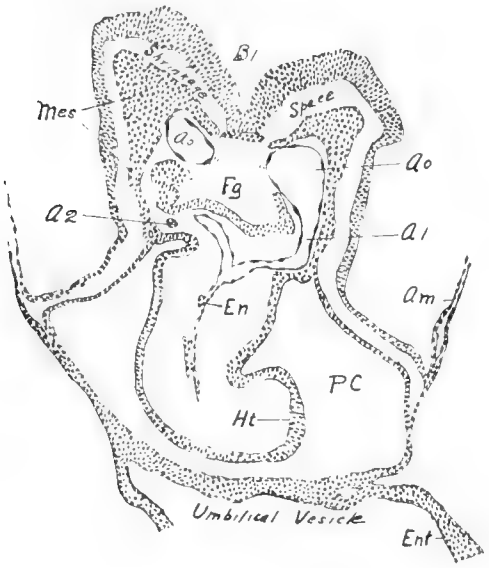
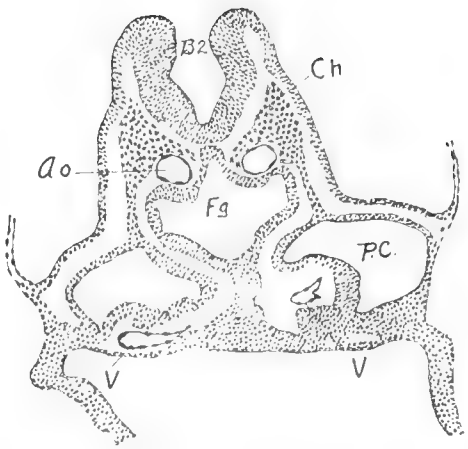


FIG. 1.



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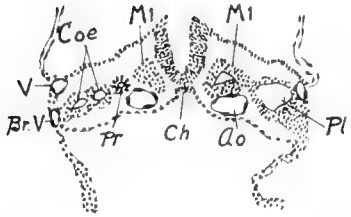
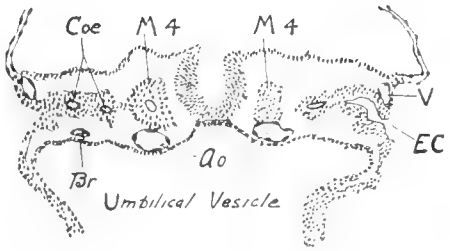


FIG. 3.



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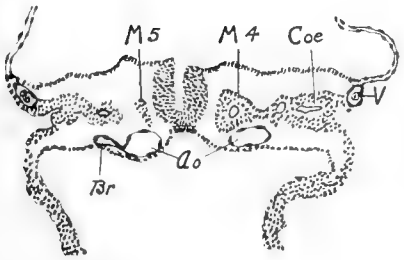


Fig. 5.

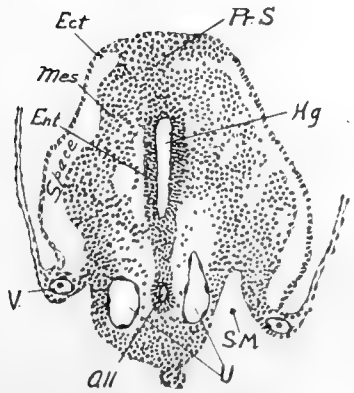


FIG. 7.

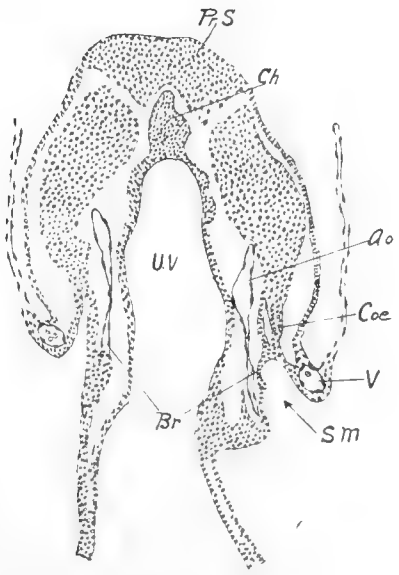


FIG. 6.

EXPLANATION OF PLATES.

Plate II (Figs. 8-11).

Fig. 8. Section 177, × 25. Outline of section to show size of the sinus formed by union of umbilical veins; V, Umbilical venous sinus (from union of the umbilical veins); All, Allantois, shown in two places, the lower is before division, the upper is terminus of long branch; Ch, Chorion; Vi, Chorionic villus, showing syncytial bud; U, Umbilical artery, shown in two places below, before union to form umbilical arterial sinus, and above, tip of sinus and large branches.

Fig. 9. Section 188, × 25. Outline of section to show size of sinus formed from union of umbilical arteries. U, Umbilical arterial sinus; All, The terminus of the short branch of the allantois; Ca, Capillaries and small branches of sinus in chorion; Ch, Chorionic Membrane; Vi, Chorionic villus, showing capillary entering.

Fig. 10. × 525. Capillaries from chorion, showing apparent formation of blood corpuscles from endothelium of capillaries.

Fig. 11. Reconstruction of cœlom, showing relation to somites; Arabic numbers on side represent the numbers of sections; Roman numerals in center represent the paired somites; P.C., Pericardial cœlom; Pl, Pleural cœlom; Coe, Peritoneal cœlom; E.C., External Communication of the cœlom; Pr, Pronephros; Ht, Projection of the heart in the pericardial cavity; X, Outer limit of body wall; Mes, Paraxial mesoblast; Br.M., Bridge of mesodermal tissue extending across the mesodermal slit; Pr.S., Primitive streak.

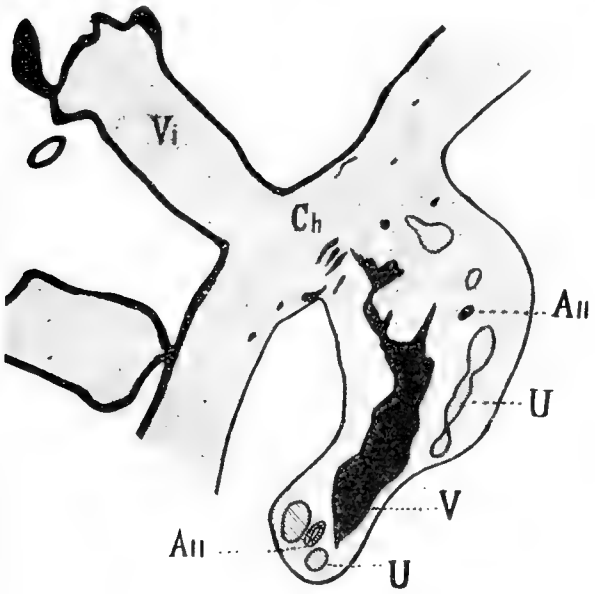


FIG. 8.

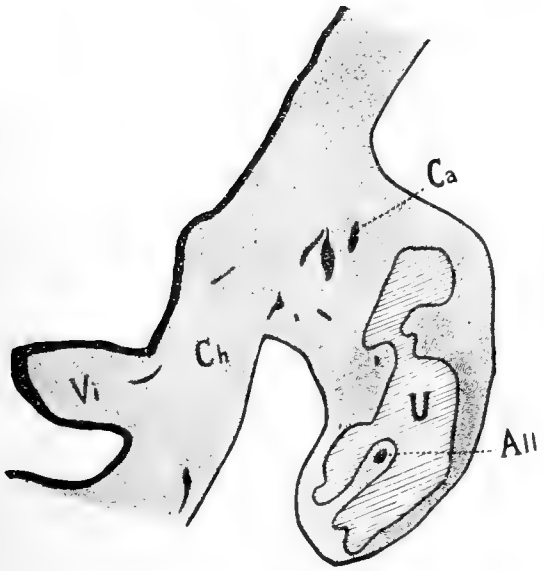
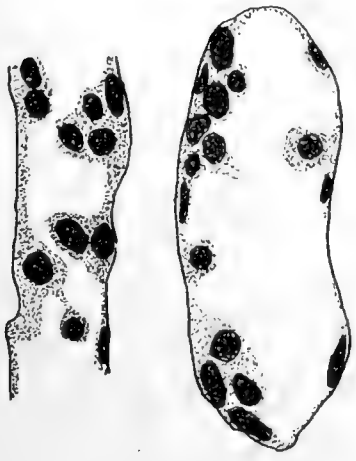


FIG. 9.



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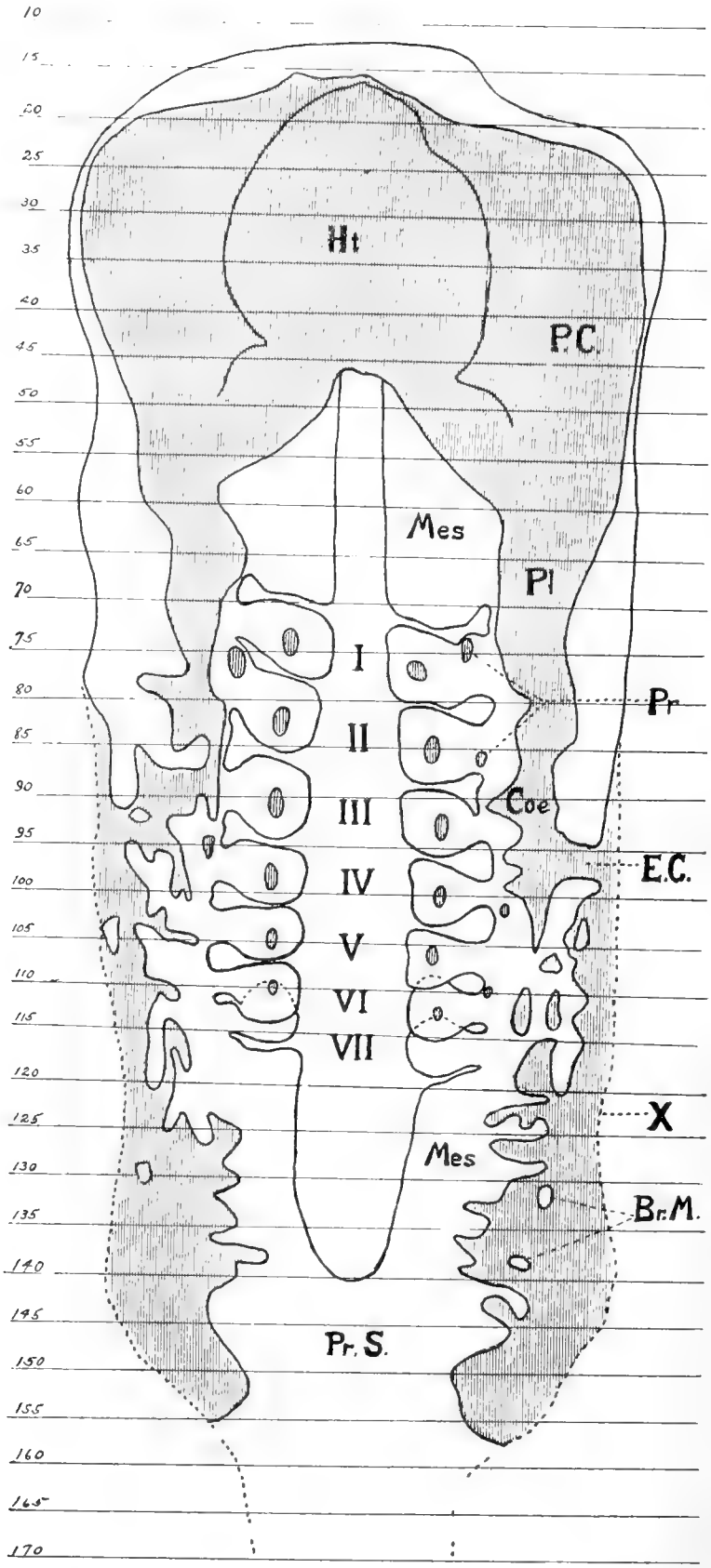
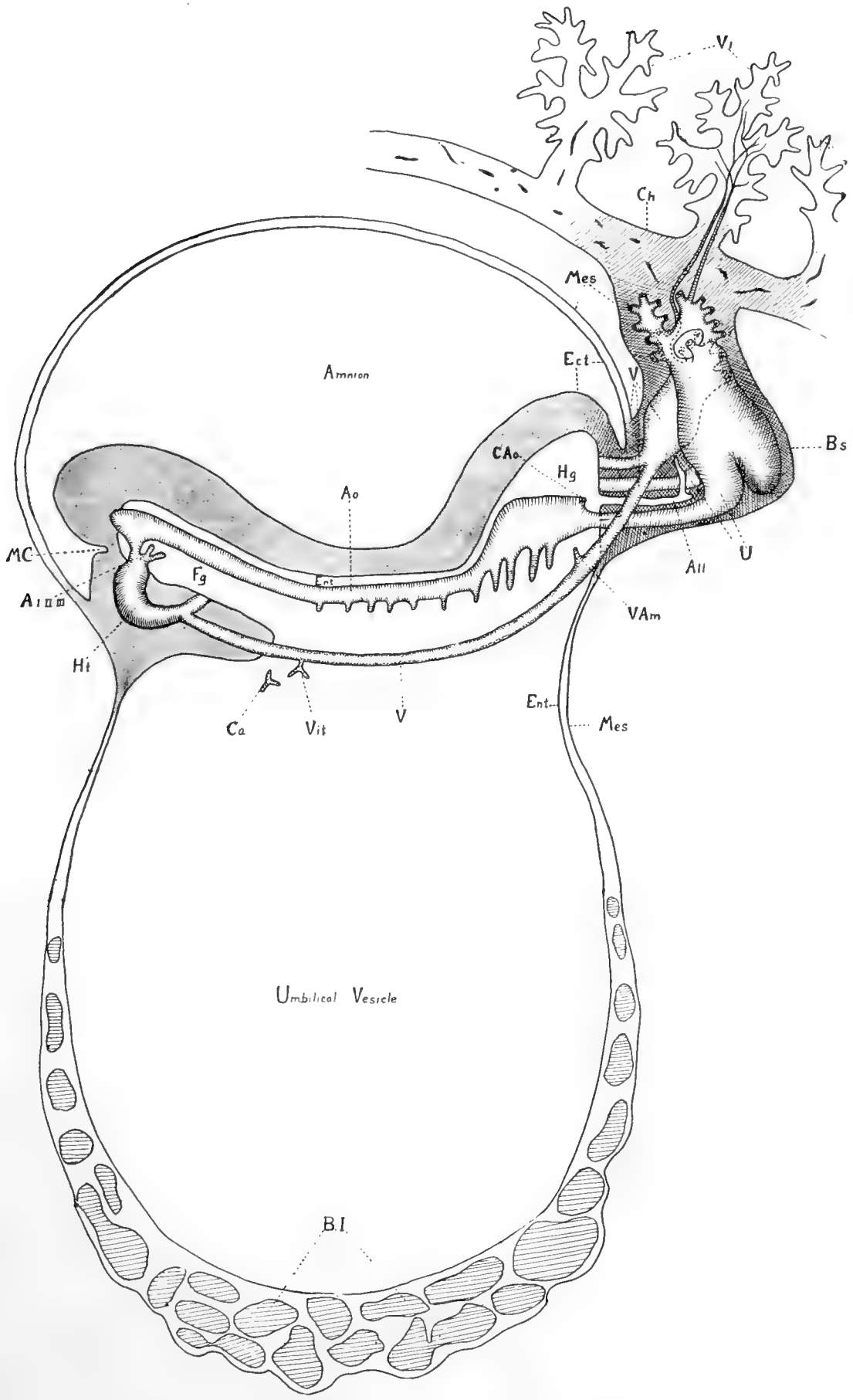


FIG. 11.

Plate III.

Fig. 12. Diagram of a sagittal view of embryo to show the vascular system. Ht, Heart; A I-II-III, First, second and third aortic arches; Ao, Dorsal aorta; C.Ao, Caudal aorta; U, Umbilical artery (unite in the bauchstiel); V, Umbilical vein (unite in the Bauchstiel); Vit, Branch of umbilical vein, is only suggestion of a possible vitelline vein; V. am, Venous branch to amnion; Ca, Capillaries; do not contain blood and do not connect with veins; B.I., Blood Islands; Ect, Ectoderm; Mes, Mesoderm; Ent, Entoderm; Fg, Foregut; Hg, Hindgut; All, Allantois; Ch, Chorionic membrane; Vl, Chorionic villi, showing vascularization; B.S, Bauchstiel; M.C., Mouth Cavity.


Vl
Ch
Mes
Ect
V
Amnion
CAo
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Bs
Ao
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All
U
Allm
VAm
Hf
Ca
Vit
V
Ent
Mes
Umbilical Vesicle
B.I

FIG. 12.

EXPLANATION OF PLATES.

Plate IV.

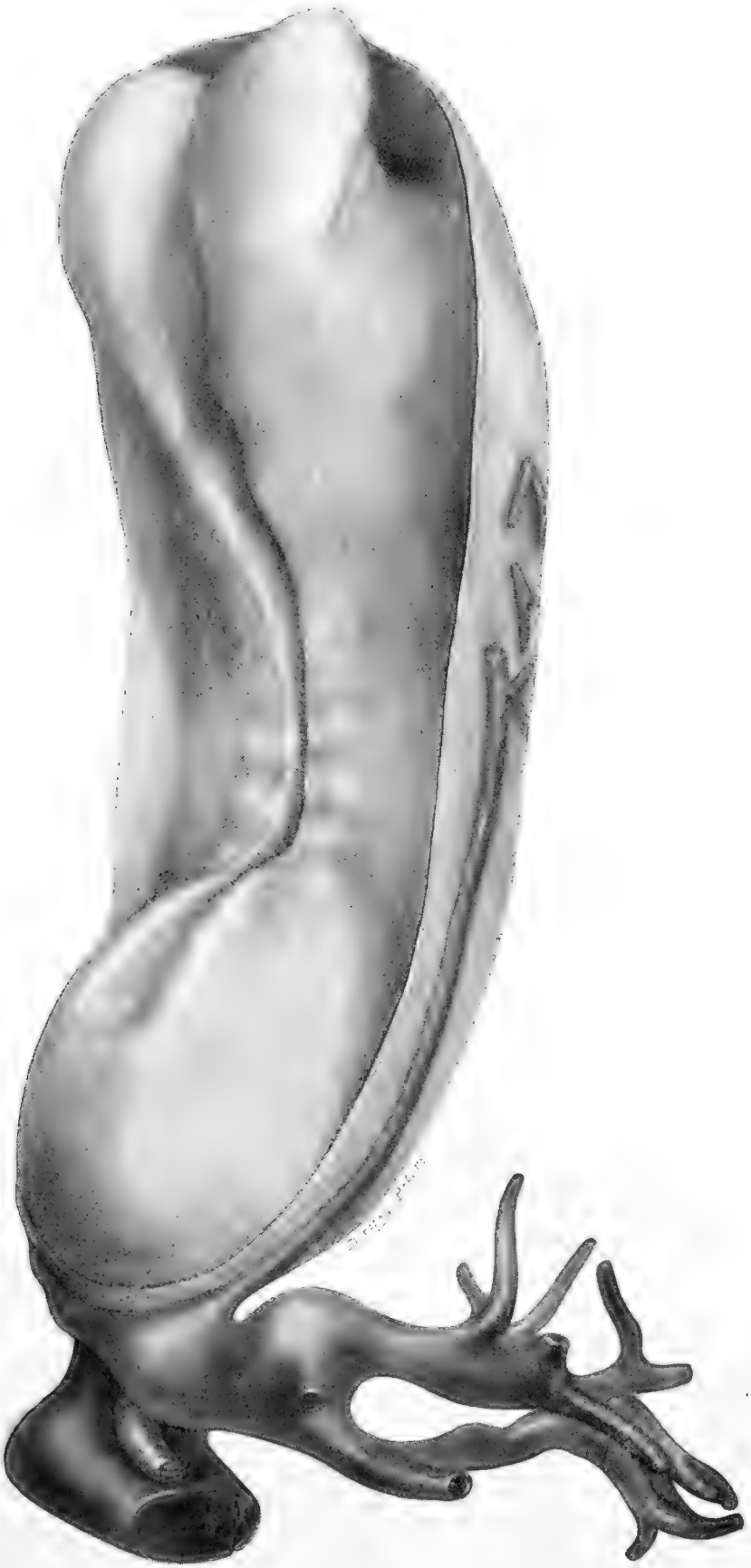
Fig. 13. Dorsol-lateral view of model of the embryo, with the amnion removed. Umbilical vein with possible vitelline branch (?) seen on right side. (The vein is projected on exterior for purpose of clearness.) Large umbilical venous and arterial sinuses posteriorly, with allantois lying between. Arterial sinus is partially cut away, venous is intact. Neural canal is open throughout, and shows the three primary vesicles of the brain.

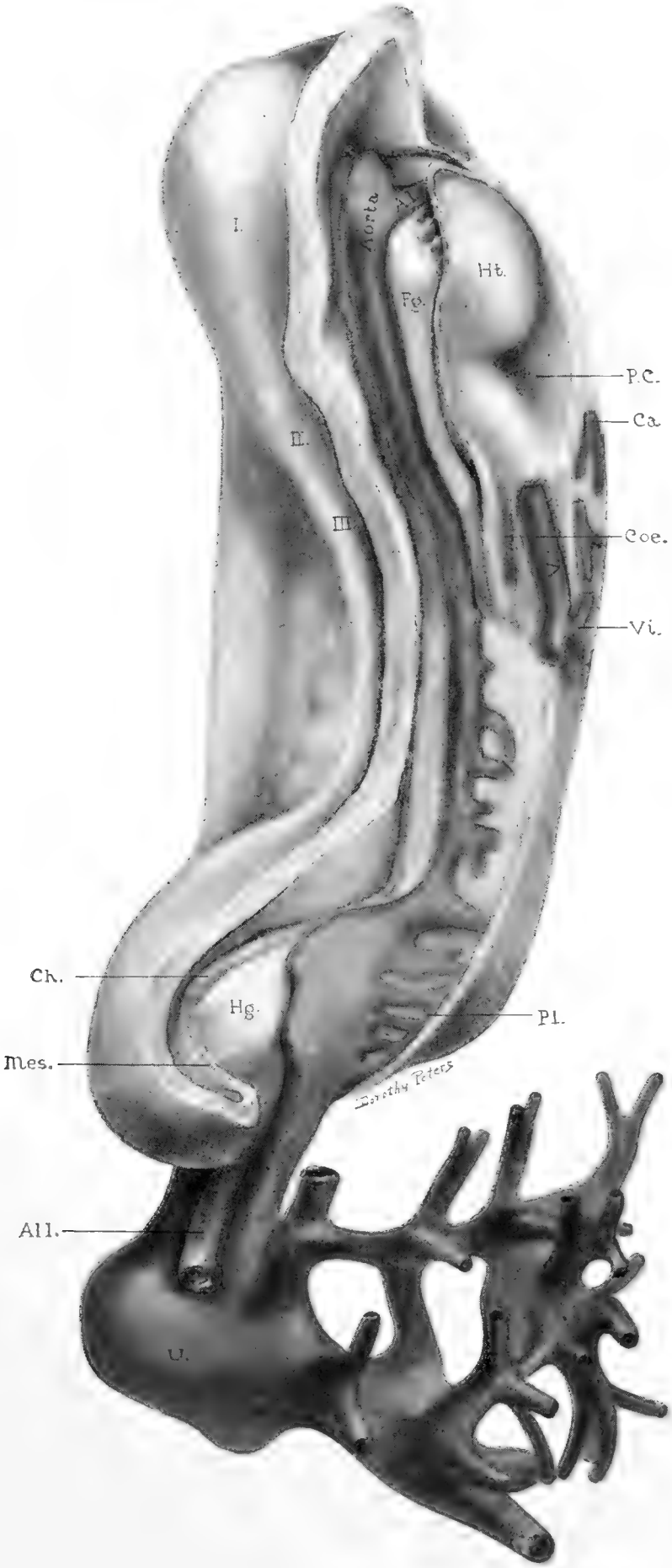
Plate V.

Fig. 14. Same view as Plate IV; window of ectoderm removed, exposing the somites and mesoderm.

Plate VI.

Fig. 15. Same view as in Plates IV and V. Mesoderm is removed. I, II, III-Three primary brain vesicles; I-First aortic arch; studs of arches 2, 3, 4, 5 are seen just posterior; U-Umbilical arterial sinus; V-Umbilical veins (umbilical veins and umbilical venous sinus have been cut away to show the arterial system). Vi, Branch of umbilical vein, only suggestion of possible vitelline vein; Fg-Foregut; Hg-Hindgut; All-Allantois; Ch-Chorda; Ht-Heart; Coe-Cœlom; P.C.-Pericardial cœlom; Ca-Capillary, but no connection; Pl-Plexus of lateral aortic branches (shown only in this place). Mes-Mesoderm in primitive streak region separated from the ectoderm by a shrinkage space, which is only present laterally, in this region. (See also Fig. 7.)




I.
Aorta
Ht.
Fg.
P.C.
Ca.
II.
III.
Coe.
Vi.
Ch.
Hg.
Pl.
Mes.
Dorothy Peters
All.
U.

A STUDY OF THE DIFFERENTIATION OF TISSUES IN THE REGENERATING CRUSTACEAN LIMB.[1]

BY

VICTOR E. EMMEL.

From the Department of Comparative Anatomy, Harvard Medical School, Boston, Mass.

WITH 8 PLATES.

CONTENTS.

[1]This paper is one of a series of experimental and anatomical studies of regeneration ('05, '06[1], '06[2], '06[3], '07[1], '07[2], '08).

Introduction.

The recent work by Reed ('04) on regeneration in the crayfish, Ost ('06) on Oniscus, and Steele ('08) on the regeneration of the compound crustacean eye, draws attention to certain fundamental questions. Their results are in marked contrast with the observations made on vertebrates where it appears that a regenerating tissue almost invariably arises from similar pre-existing tissue. The important relations of this subject to the problem of both the normal and pathological origin and cytomorphosis[2] of new tissue cells, render it desirable to investigate further the histogenesis of regenerating tissues. The present paper contains the results of a cytological study of the cells and tissues in the regenerating chela of the lobster, a crustacean which has been especially favorable for this study, both on account of its highly developed capacity for regeneration and because of the unsurpassed opportunity for obtaining suitable material at the lobster hatcheries.

The results to be described deal more especially with (1) the cytological changes observed in the regenerating ectodermal cells; (2) the genesis of striated muscle, nerve, and connective tissue; and (3) the morphology of certain structures at the breaking joint of the limb, which as far as the writer is aware, has not previously been considered.

I desire to express my thanks to Professor C. S. Minot, of the Harvard Medical School, for interest and encouragement during the

[2]This term is used "to designate comprehensively all the structural modifications which cells or successive generations of cells may undergo, from the earliest undifferentiated stage to their final destruction." See Minot ('01, p. 494.)

progress of the present work. I am also indebted to Professor A. D. Mead, of Brown University, for generously permitting me the use of materials and apparatus at the Experiment Station of the Rhode Island Commission of Inland Fisheries; and to Professor R. J. Terry, of Washington University, for valuable suggestions during the completion of the present paper.

II. Material and Methods.

A serious obstacle encountered in determining the origin and differentiation of a regenerating tissue is the difficulty of securing a complete series of successive developmental stages. If a number of animals are operated upon at the same time and material fixed at successive intervals, it is usually found necessary in subsequent study to make an extensive rearrangement of the material on account of variations in size and rate of growth. These technical difficulties were considerably lessened in the present work because of the excellent material obtained at the lobster hatchery of the State Experiment Station at Wickford, Rhode Island. From a hatchery pool containing several thousand lobster fry, it was possible to select a large number of animals which were practically equal in age and size, and which had all moulted to the same stage within less than twenty-four hours. This last point is of considerable importance, since it had been ascertained in previous experiments ('06) that the age of the animal and the time, in relation to moulting, at which the operation is made are important factors influencing the rate of regeneration. These young lobsters were also further favorable for study because the exoskeleton had not as yet attained such an amount of chitin and lime salts as to necessitate decalcification before sectioning.

The operation consisted in the autotomous removal of both chelæ. The lobsters, about 300 in number and all practically equal in age and size, were placed in floating cars and kept in as nearly normal condition as possible. Specimens were then preserved at two, four, and eight hour intervals throughout a period of fourteen days, in which time the chelæ had undergone complete regeneration. From this succession of stages about 54 were selected, sections of the chelæ were cut 5 micra in thickness and mounted in series. In order to

facilitate a comparative study, the specimens were all cut in a nearly constant plane. In addition to this series, sections of the regenerating limbs of older lobsters were also studied.

The material used was fixed in a saturated 35 per cent alcoholic solution of corrosive sublimate. Several other fixing agents were tried, but all solutions containing acids proved unsatisfactory on account of their injurious reaction with the chitin and salts of the exoskeleton. The nuclear and cytoplasmic stains employed were alum hæmotoxylin, Heidenhain's iron-alum-hæmatoxylin, Congo-red, eosin, and Mallory's connective tissue stain.

III. Anatomical Structure of the Chela.

1. *Segments, Muscles, and Joints.*—The following account is confined to those structural characteristics of the limb which are essential for the subsequent description of its regeneration.

The chela of the lobster is composed of six segments (Fig. 37). Proceeding in a disto-proximal direction these segments are known successively as a dactylopodite or dactyl (*d*), propodite (*p*), carpopodite (*c*), meropodite or meros (*m*), ischipododite (*i*), and basipodite (*bs*). A seventh basal segment not involved in the present study is the coxopodite. The index is the distal part of the propodite which opposes the dactyl and forms one jaw of the large claw. All of these seven segments are united to each other by flexible joints with the exception of the last two,—the ischiopodite and basipodite. These are fused together into an immovable joint or "breaking-joint," a structure especially adapted to the process of autotomy. Each joint is like a hinge, articulating at two points and permitting only a simple flexion and extension of the segment. The musculature of the chela consists of a pair of opposing muscles for each of the segments with the exception of ischiopodite (Fig. 37). The pair of muscles in any given segment is attached proximally to the chitin of the proximal region of the segment. Distally the converging muscle bundles are attached to a chitinous plate or ingrowth of the exoskeleton of the next segment distad. The origin and insertion of the extensor and flexor are on opposite sides of the joint. In each of the first three pairs of muscles, the flexor is larger than the opposing extensor.

The ischiopodite is an exception to the above description, for the muscles are not arranged to act in opposition. There are two muscles in this segment, but they both have their origin on the same side of the limb, *i. e.*, on the ventral side of the fourth joint (Fig. 37, e^4). One muscle is slightly larger than the other, but both are smaller than any other muscles of the chela. The larger muscle is inserted partly on the morphologically anterior side and partly on the dorsal surface of the exoskeleton in the proximal part of the ischiopodite. The smaller muscle is inserted directly on the morphologically anterior wall of the segment. Both muscles, therefore, appear to function as extensors. A similar arrangement of muscles has been described by Fredericq ('92) in the ischiopodite of the crab. That a similar condition exists in the crayfish is not so clear, however, for Reed ('04) states that the ischiopodite of the cheliped in the crayfish possesses "both an extensor and a flexor" (p. 310).

2. *The "Breaking Joint."*—a. *Exoskeleton.*—By the breaking joint (Fig. 37, *bk*) is meant the region in which a fusion has taken place between the ischiopodite and the basipodite. Since it is at this region that the chela is amputated in autotomy and the new limb begins to regenerate, a more detailed description of the breaking plane or joint is essential for our present purpose.

The external appearance of the breaking joint in the adult has been described by Herrick ('95) as a "fine hair line leading from the small spur next to the articular facet on the under side, round the anterior border to the upper side of the joint. It then bends forward and abruptly backward, crossing the small proximal end of the joint, to near its point of departure" (p. 10). This hair line represents the plane of union of the basipodite and ischiopodite into a solid compound segment. In the first three larval stages there is a movable articulation between these two segments and as described by Herrick, "there is no true fusion of the segments until after the fifth stage" (p. 102). It may be questioned, however, whether there is any longer a functional articulation of these segments even during the fourth and fifth stages of development. Although externally the joint may present the appearance of a free articulation as indicated in Herrick's figures, the examination of longitudinal sections

of the fourth stage limb shows a different condition. As may be seen in Figs. 1 and 2, there is strictly no free articulation evident, especially on the morphologically ventral side (see also Fig. 3, *bk*), where there is an almost perfect continuity of the exoskeleton from one segment to the other. On the dorsal side the union is less complete. A difference between the two sides of the breaking joint is evident even in the adult where the chitin is still much thicker on the ventral than on the dorsal surface. In section the region of the breaking joint can easily be identified by the characteristic coloration which the chitin takes with Mallory's connective tissue stain. With this reagent the exoskeleton, with the exception of the outer lamellæ of chitin, takes on a bright blue color, whereas the line of fusion between the two segments is sharply differentiated by becoming dark red.

The internal structure of the breaking plane of the limb may be described as follows. The epithelium (epidermis or "hypodermis") of the exoskeleton is continuous across the breaking plane, from one segment to the other. The connective tissue occupies the interior of the breaking joint and is complex in its arrangements. Certain structural relations of this tissue were discovered, which differ considerably from those previously recorded. They will presently be described.

It can be readily appreciated that the mechanism for preventing an excessive loss of blood is an important element in the perfection of a breaking joint adapted to the autotomous removal of the limb. Such a mechanism is supplied by the connective tissue structures. Fredericq ('92) was among the first to describe the separation of the blood cavities of the basipodite and ischiopodite by a "cloison membraneuse" or circular diaphragm, stretched across the distal part of the basipodite (p. 177). Similar descriptions have been written by Andrews ('90) for Libina, and by Herrick ('98) and Reed ('09) for the lobster and the crayfish. This transverse membrane was at once interpreted as a structure for preventing hemorrhage after autotomy. Andrews describes the membranous fold in the breaking joint of the spider crab as extending "from the epidermis to the central nerve and blood vessels," and states that after autotomy the membrane covers

the entire surface of the stump except at the centre, "where there is a rounded hole with a little torn tissue and blood exposed". Reed states that in the lobster and the hermit crab "the opening through the membrane for the nerve and the blood vessel is in about the centre of the exposed surface when the leg is thrown off" (p. 309). In an earlier description of the lobster, the writer ('05) also described this membrane as "extending almost entirely over the basipodite" and "perforated only at the centre by an artery, the blood sinus, and a large nerve" (p. 89). It was with considerable surprise, therefore, that it was found in the present study of serial sections of the chela that the above descriptions cannot, for the lobster at least, be regarded as strictly accurate, particularly in regard to the relation of the blood sinuses.

In order to obtain a more thorough conception of these structural relations, graphic reconstructions of the breaking joint have been made from serial sections of the right chela of a fourth stage lobster. The tissue structures of the breaking joint at this stage are perhaps somewhat simpler than in the chela of an older animal, but their essential relations are, I believe, practically the same. It should also be stated that these reconstructions are in one detail not complete, since the connective tissue network in which the arteries and nerves are partly embedded as they pass along the walls of the large venous blood sinuses, is not represented in its entirety. Figs. 1 and 2, respectively, show the outer and inner, or morphologically posterior and anterior, parts of the breaking joint and adjacent segments. Fig. 3 represents a median section between the two parts shown in Figs. 1 and 2. The latter figures do not show quite half of each side.

b. *Blood Vessels and Nerves.*—It may be observed in these figures that there are two nerves, two arteries, and a large venous sinus (divided into two separate channels) which pass through the region of the breaking joint. The two nerves n^1, n^2, lie on the inner or medial side of the joint, the larger nerve (n^1) giving off a small branch as it leaves the basipodite. The main artery (a^1) passes along the outer wall. In the basipodite it gives off a small branch (a^2) which takes an oblique course across toward the inner wall of the ischiopodite. The venous blood of the chela is carried toward

the body through an immense blood sinus (*vs*). As this sinus passes through the ischiopodite it is temporarily separated into two channels by a connective tissue partition or septum (*S*). One of these channels passes ventrally and the other dorsally, through the breaking joint. The two nerves and arteries lie within the septum and adjacent connective tissue separating the two venous channels.

In the course of these two blood channels just as they enter the basipodite after having passed through the breaking joint, there occur two very interesting valve-like folds of connective tissue. These two structures arise in the following manner from the median septum separating the blood channels. As the septum extends proximally into the basipodite it becomes divided into two plates or folds (v^1, v^2) much like the arms of an inverted "Y," one of the arms passing toward the dorsal wall and the other toward the ventral wall of the segment. The fold extending toward the dorsal wall is somewhat shorter and thicker than the one on the ventral side. These two folds do not completely unite with the walls of the basipodite. A part of the border of each fold retains a free edge, which, together with the opposing wall of the segment, completely encloses the lumen of each blood channel. Beyond (*i. e.*, proximally) these folds the two blood channels reunite to form again a common venous sinus (vs^2).

While endeavoring to understand the significance of these folds as they were first seen in the serial sections, it was suggested that they might possibly function as valves for the venous blood channels, the valves closing when the limb had been autotomously removed. Following up this suggestion, serial sections of specimens which had been fixed immediately after the autotomy of the chela, were then examined. It was at once evident that the folds in question had assumed in these specimens a position different from that observed in sections of a chela before the limb had been removed. Each fold had now become distended in a distal direction as if caught by an outward blood pressure, the result being the complete closure of the ruptured end of the two blood channels (Fig. 4, v^2). Desiring to make a still further test, a number of live lobsters were then obtained and the chelæ autotomously amputated. At first there was a short

jet of blood from the exposed stump, and then a slight bulging of the tissues over the surface of the stump with no further escape of blood. The location of the two venous channels could be distinguished quite readily by the transparency of the now turgid valves closing each lumen. The two valves were then gently pressed inwards with the tip of a pencil and in each case, a slight opening of the valve being effected, there at once followed a jet of blood. The valvular function of these folds seems, therefore, to be clearly demonstrated.

This description may be summarized with the statement that a septum of connective tissue extends across the cavity of the limb in such a way as to divide the venous sinus into two channels in its passage through the breaking joint. Through this septum and the adjacent connective tissue pass the two arteries and nerves. Proximally the septum forms two folds which function as valves for the venous channels.

It can readily be appreciated how in previous descriptions some of the relations of these structures may have been overlooked. For when the valves have closed after autotomy, the remaining stump presents every appearance of a continuous membrane extending over its exposed surface, "perforated only by a nerve and a blood vessel," so that in the following statement Fredericq ('92) even indicates a doubt regarding the passage of the vein, "Seul le nerf mixte de la patte, l'artère (et un sinus veineux?) franchissant cette limite et passent du basipodite dans l'ischiopodite, à travers un orifice étroit, creusé dans la membrane obturatrice" (p. 177).

c. *An Embryonic or Transitory Muscle.*—Both Fredericq ('92) and Morgan ('00) have recorded the fact that in the chela of the adult crayfish or lobster "no muscle passes from the ischiopodite to the basipodite". In other words, in the adult lobster no muscle extends across the breaking joint. It was with considerable surprise, therefore, that it was found that this is not true for the larval lobster. In the study of sections of the chela of larval animals, a well developed muscle was discovered extending from the ischiopodite to the basipodite, and consequently passing through the breaking joint. This muscle is easily seen in a longitudinal section of the limb (Fig. 5, *m*). It is attached to the inner side of the ischiopodite, passes

through the ventro-anterior region of the breaking joint, and is inserted upon the inner wall of the basipodite. It functions as a flexor. This muscle persists throughout the first four larval stages of the lobster's development. During the fifth stage, however, it begins to degenerate, and at the sixth stage the former muscle is represented by a mere bundle of connective tissue (Fig. 6, *ct.*), containing a few remnants of the degenerating muscle fibers (*md*).

It is interesting to note that the stage of the lobster's development at which the degeneration of this muscle occurs is not only the one just preceding the stage in which the asymmetrical differentiation of the chela first becomes evident, but, as the writer ('08) has previously shown, it is also the stage after which the asymmetrical differentiation of the chelæ can no longer be experimentally controlled.

The discovery of this muscle introduces a new fact to be taken into account in the question of the morphological significance of the breaking joint. Andrews ('90) maintains that the origin of the breaking joint may be "explained as a modification of a former free joint" (p. 142). On the other hand Reed ('04) concludes, from a comparative study of the crustacean limb, that it "seems, therefore, erroneous to state that the breaking joint corresponds to the lost joint" (p. 311). Without entering into a discussion of this question, it may be observed that the existence of a muscle in the chela of the larval lobster which crosses the breaking joint and is attached to its two adjacent segments, seems a strong point in favor of Andrew's conclusion.

d. *Autotomy.*—The process of autotomy or defensive mutilation among crustaceans has been fully described by Fredericq and Morgan. When the nerve within the chela is experimentally stimulated a vigorous contraction occurs in the muscles of the basal segment, followed by a sudden snapping off of the limb at the breaking joint, leaving the exposed surface of the stump as smooth as if cut with a keen-edged knife. Regarding autotomy in the larval lobsters, I can confirm Herrick's observation that the "casting of the claw" does not occur before the fourth larval stage. In experiments with several hundreds of first, second, and third stage lobsters in which one or both chelæ were removed, I have never observed the limb to

be thrown off autotomously, although under a slight tension, such as may be produced by a gentle pull with a small forceps, the chela will, however, usually part in the region between the basipodite and ischiopodite, *i. e.*, at the joint which is destined to develop later into the breaking joint of the adult animal.

IV. Early Stages of the Regenerating Limb.

1. *The Readjustment of Injured Tissues.*—Fig. 4 represents a longitudinal section of the stump of the right chela just after autotomy. The valve (v^2) has been carried outwards as the result of the amputation and now closes the venous channel *vc*. The exposed edge of the layer of epithelial cells of the epidermis (*e*) projects slightly above the level of the broken chitin (*ch*) of the stump. Shortly after autotomy the vascular cavities at the distal end of the stump become filled with a dense mass of nucleated blood corpuscles (Fig. 7, *bc*). The muscle (Figs. 2 and 5, *m*), which was torn by the operation, degenerates and is not restored again in the later regeneration of the limb, a result which might be expected, for it will be recalled that normally this muscle degenerates during the fifth stage and is absent in the adult lobster.

A protecting crust of coagulated blood plasma and blood corpuscles soon becomes evident over the exposed surface of the stump. This blood clot (Fig. 7, *bcl*) forms above, or in other words distal to, the connective tissue membrane extending across the limb. The relation of this blood clot to the connective tissue membrane differs somewhat from that described by Reed ('04) as occurring in the crayfish, where the "blood cells form a plug at the opening through the membrane, and collect in a thick layer just beneath the ectoderm cells, which form the proximal layer of the membrane of the breaking joint. . . . After a time these blood cells arrange themselves in horizontal lines crowded close under the ectoderm cells of the membrane." Reed further states that "in a few hours from the time that the leg is thrown off the membrane takes on the appearance of dead tissue. . . . The blood cells soon degenerate, and most of them are thrown away with the membrane, when this is later cast off" (p. 312). In contrast to this, as may be observed in Figs. 4

and 7, the corresponding membrane of the chela of the lobster is composed only of connective tissue and does not appear to degenerate to any appreciable degree. On the contrary, in the lobster the original connective tissue membrane and the valves for the venous sinuses persist throughout the regenerative activities, and retain their original function in the new limb. In Fig. 7 it may be seen that as the regenerating epidermal cells begin to migrate across the stump they pass between the outer blood clot (*bcl*), and the connective tissue membrane and the venous valves. The venous valves no longer exerting a resistance to the blood stream, now begin to assume their original and normal position (Figs. 7 and 8). There is little, if any, degeneration of the connective tissue. In the blood clot, however, the corpuscles soon deteriorate, the nuclei become flattened in the disto-proximal direction of the limb, and both the nuclei and the cytoplasm of the clot assume a deeper stain with hæmatoxylin and Congo red.

2. *Migration of Epidermal Cells.*—As the blood clot forms, the epithelium of the exoskeleton retracts slightly within the stump. There is, however, no other noticeable change in the position of the epidermal cells until after the fourteenth hour following the operation. The first regenerative activity which is then observed is a migration of ectodermal cells from all sides of the epidermal wall. This movement of the epidermal cells appears at first to be more pronounced in the region of the limb nerves on the inner side of the stump; in a section taken through the outer side of the stump twenty-four hours after autotomy (Fig. 7) the migrating epidermal cells (e^1, e^1) from the opposite sides are still quite widely separated, but in a section from the inner side of the stump the migrating cells are found much nearer each other. These cells continue advancing centripetally until they unite to form a complete layer of epidermal cells over the surface of the stump. During the migration, the congested mass of blood corpuscles within the distal end of the stump begins to disintegrate, but the old connective tissue remains intact as already described.

3. *Nuclear Changes in the Epidermal Cells.*—The epidermal epithelium consists of low columnar or cuboidal cells, apparently

fused into a true syncytium. Indeed, Huxley's description of the epidermal epithelium of the crayfish applies equally well to the lobster,—"It is found to consist of a protoplasmic substance in which close set nuclei are embedded . . . there can be no doubt that it is really an aggregate of nucleated cells, though the limits between the individual cells are rarely visible in the fresh state" (p. 178). It may be added that in the lobster at least, cell boundaries are no more clearly defined even after fixation and staining. In view of these facts it becomes evident that in making observations upon cell changes in such a tissue, attention is necessarily more largely directed toward the nuclei of the tissue cells.

The characteristic structure of normal epithelial nuclei as they appear immediately after the autotomy of the limb is shown in Fig. 11. They may be described as more or less elongated or oval in form with a clearly defined nuclear membrane. A structural characteristic is the segregation of the chromatin into relatively large angular masses or karyosomes. These karyosomes take on a heavy stain with hæmatoxylin, and are rather evenly distributed throughout the periphery of the nucleus. The ground substance stains a uniformly light color. The four nuclei in Fig. 11 were from the epidermal epithelium just below the breaking plane and from a region at the inner side of the basipodite near the limb nerves.

Fig. 12 represents epidermal nuclei fourteen hours after autotomy. To facilitate a more direct comparison of structural characteristics, these nuclei were taken from practically the same region as the nuclei shown in Fig. 11, *i. e.*, from the inner side of the basipodite,—a region of the epidermis where regenerative activities are first observed. Of these four nuclei, *a* and *b* are proximal of *c* and *d* and farthest from the region of the first regenerative changes. In contrasting *c* and *d* with *a* and *b*, and also comparing with Fig. 11, it may be noticed that the nuclei are becoming larger in size and the chromatin is undergoing certain changes. In *a* and *b* the karyosomes are still large and are similar to those in Fig. 11. But in *c* and *d* the chromatin is more finely subdivided and arranged in a reticulum of chromatin strands and knots. In nuclei *a* and *c* a round, centrally located body is seen, which takes a relatively lighter stain and has the

characteristics of a nucleolus. Whether the presence of nucleoli is typical of this stage is, however, questionable, for with the same stain they can also be found, although less frequently, in the normal or resting nuclei.

The nuclei shown in Fig. 13 are from a limb fixed twenty-four hours after autotomy. At this time the migrating epidermal cells have progressed considerably in their advance over the surface of the stump. The nuclei drawn are from the same specimen as are those of Fig. 12, but they have been selected from a region where the advancing epithelium has already partly closed over the inner side of the stump. As the epidermal nuclei migrate, their long axes rotate through an angle of about 90 degrees from their former position, and in such a way that at the center of the stump the long axis of the nucleus assumes a position practically parallel to the long axis of the limb (Fig. 8). In Fig. 13 the nuclei *c* and *d* are nearer the center of the stump. As the nuclei approach the central region, there is an increase in their shortest diameter, giving each nucleus a broader and less elongated form. They now also become more widely separated from one another. In the earlier stages of migration the lower or proximal ends of the nuclei tend to assume a tapering or pointed form.

A striking structural characteristic is the unequal distribution of the chromatic material (giving the nuclei a "loaded" appearance). The proximal end of each nucleus takes a relatively deep stain, while the upper or distal end of the nucleus is so pale that frequently little or no stain can be detected except in the few scattered granules of chromatin. In nuclei *a*, *b*, and *d*, one can see that the boundary between the light and dark areas, or zones, lies at such an angle across the nucleus that the darker zone occupies the lower and left region, and *vice versa* for the lighter zone. The nuclear contents have much the appearance of having been centrifuged. In nucleus *c* the plane separating the two zones is more nearly equatorial in position. It will also be recalled that these nuclei were taken from the left side of the section of the limb (*c* being nearest the center of the limb). In nuclei taken from the opposite or right side of the same section, it is interesting to find the localization of the two zones just reversed, *i. e.*, the

more lightly stained area is now at the upper and left side, whereas the darker zone at the proximal end is directed toward the right side of the nucleus. In studying the sections, a considerable enlargement or expansion of the nucleus is frequently found at the distal end, or region of the light zone, in some cases being even more marked than is shown in nucleus *d* (Fig. 13). At this stage of migration the former karyosomes have now almost disappeared. The chromatin has disintegrated into fine granules, which appear to be either peripherally distributed or axially segregated into a dense mass. It is also to be observed that the denser central mass is always located at the darker pole of the nucleus.

The significance of this polarization of the nuclear contents (if it may be thus designated) is not readily apparent. It can hardly be considered an artifact resulting from faulty fixation or staining. On the contrary, its regular occurrence, its reversal at opposite sides of the limb, the frequent expansion of the lighter pole, the association of the axial chromatin mass with the darker pole, together with the fact that similar conditions are even more conspicuous in later stages of the regenerating limb as will be presently described, is evidence which supports the conclusion that this polarization of nuclear contents is a characteristic structural feature of certain phases in the regenerative activity of the epidermal cells.[3]

Rand's ('04) discussion of the regenerating epidermis of the earthworm applies equally well to the migrating epidermal cells in the regenerating lobster's limb. He says: "The movement of the cells at the margin of the layer is not a passive one, resulting from external pressure. Nor is there the slightest ground for supposing the existence of a force acting from a position anterior to the cut epidermal edges and serving to pull the epidermal cells over the cicatrix. We are compelled, therefore, to look in the individual epidermal cell itself for the immediate source of the activity. This activity, by whatever mechanism effected, must be occasioned by an agency external to the cell, viz., by some factor of the conditions resulting from the injury." Beyond this we must admit that as yet we

[3]The resemblance of the "polarized" nuclei in appearance to certain stages of synapsis of the nuclei in spermatogonia may be noted, although it has suggested no further explanation of the "polarization."

have not succeeded even in determining whether the stimulus thus controlling the cell activity is some "chemical peculiarity" of the injured and exposed tissue, or whether it is some "inter-action" of the epidermal cells, questions which are merely preliminary to understanding the factors which may account for the apparent polarization of nuclear contents just described. These nuclear changes might possibly be classed among the phenomena designated as "cytotaxis" (Roux, '96), although in doing so it is not evident that we advance materially in a solution of the problem. At the end of the next fourteen hours, *i. e.,* thirty-eight hours after autotomy, the epidermal cells have spread completely over the stump and the first mitotic cell division appears. During this period the nuclei have assumed a more spherical form and appear somewhat larger. Both the nuclear sap and chromatin have become more equally distributed. The chromatin is now more coarsely granular, and as the nucleus approaches the prophase of mitosis, the chromatin collects in conspicuous rod and knot-like masses or chromosomes (Fig. 14, *a*). The nuclear membrane then disappears and the cell passes through the various mitotic phases, of which a later metaphase is shown in Fig. 14, *b*.

The volumetric increase of nuclear material during the initial regenerative activities presents another fact for consideration. In many respects the problem arising from the phenomena of cytomorphosis and regulation in the reproduction of a part of the organism are comparable with similar problems in the development of the original embryo. In both processes the differentiation of anatomical structure is preceded by the formation of a more or less undifferentiated mass of cells, characterized, in the case of embryonic development, by the segmentation of the ovum, and in the case of regeneration, by the proliferation of a mass of cells from the tissues in the region of injury. While the present data hardly justify an extended discussion, attention may, however, be called to the fact that in both regenerative and ontogenetic processes, the initial cytological changes are attended by a characteristic increase in the amount of nuclear material.[4]

[4]It should be observed that in the case of regeneration, at least in the earlier stages, the increase of nuclear material apparently is not associated with cell division.

In regard to the segmentation of the ovum, Professor Minot ('08) emphasizes this increase in nuclear material as a characteristic feature of "the process of rejuvenation," and as a result of his studies of the structural relations of cytoplasmic and nuclear material during the development of the organism, concludes "that as we define senescence as an increase and differentiation of the protoplasm, so we must define rejuvenation as an increase of the nuclear material," p. 167. Whether we shall be justified in attaching a similar significance to the corresponding nuclear changes in regeneration, or whether in the latter case the nuclear phenomena are fundamentally different in nature, are questions for further investigation.

4. *Cytoplasm.*—In describing the cytoplasm of the epidermal cells, it is necessary to consider the cells collectively, on account of the ill-defined or even non-existent cell boundaries. In the normal or resting cell the cytoplasm has a reticular structure of rather even appearance and is traversed by larger delicate supportive fibrils lying more or less parallel to the long axis of the cell. As the epidermal cells migrate over the surface of the stump, their cytoplasm ceases to be characterized by the presence of fibrillar structures, but appears to be composed of fine granules rather uniformly distributed (Figs. 8 and 9). A definite reticular structure is no longer evident. The inner surface of this sheet of migrating cells, instead of being smooth, presents an uneven contour due to numerous rounded inward projections of cytoplasm. These projecting masses of cytoplasm never show definite limiting membranes, but their boundaries can, however, be traced upward into the general layer of cytoplasm as far as the level of the epidermal nuclei, the relations of each mass being such as to indicate that it represents the unit of cytoplasm coming under the influence of each nucleus. The volume of cytoplasm appears to have increased, as is indicated by the wider separation of the nuclei and the greater depth of the epithelium after its migration has been completed.

One result of these observations on the epidermal cells is to emphasize the fact that in the migration of these cells as the first step in regeneration, there occurs a parallel series of definite structural

changes in cytoplasm and nucleus. It remains for further observation to answer the interesting question whether the parallel occurrence of these structural changes is due to the existence of a significant correlation between the nucleus and the cytoplasm in the regenerative activities of the cell. Such a correlation is supported, for example, by Eycleshymer's ('04) thorough work on the muscle cell of Necturus, in which that investigator finds strong evidence that the nuclear material plays a most important rôle in cytoplasmic synthesis. He suggests that "cellular degeneration and regeneration are accompanied by volumetric, structural and chemical changes in chromatin" (p. 307).

5. *Cell Division.*—Reference has already been made to the fact that mitotic cell division was not observed until the second day following the operation, and then only in the epidermal cells. Thirty-eight hours after the amputation, two mitotic figures were found in serial sections of the right chela and four figures in the left chela of the same specimen. Twelve hours later, eighteen mitotic figures were counted. Preceding mitosis the nucleus migrates toward the outer surface of the epidermis so that the mitotic figures are always found near the periphery of the epithelium. Regarding the question of amitosis in the early stages of regeneration, it may be said that there was no evidence found for direct or amitotic division of the epidermal cells during the thirty-eight hours preceding the first mitosis. Each migrating nucleus seems to maintain its original unity up to the time at which mitosis first occurs.

Without entering further into a discussion of this interesting question of amitosis, attention may be called to the contrast of the present results as compared with observations on the compound crustacean eye, where it appears that in "all cases of the regeneration of the eye the nuclei are increased by amitotic division" (Steele, '08, p. 183). Steele, referring to Reed's ('04) conclusion that mitotic figures do not occur in the early stages of the regenerating limb of the crayfish, suggests (p. 184) that amitotic division may possibly have taken place "during the preparatory stages at least." In regard to this question, the evidence, in the lobster at least, is of a negative character. It appears rather that an increase in both the quantity of cytoplasm and

the size of the nuclei, together with their wider separation during migration are sufficient to account for the formation of the first epithelium over the injured surface of the limb.

Similar preliminary regenerative conditions have also been observed in other animal forms. Among the vertebrates, Barfurth ('90), from a study of regeneration in the amphibian tail, concludes that the new cells which first cover the wound "stammen her vom persistierenden Epithel der Wundränder, sind nicht etwa durch Theilung aus diesen Epidermiszellen hervorgegangen, sondern haben sich aus dem Epithelverbande losgelöst, sind embryonal beweglich (amöboid) geworden und schieben sich langsam über die Wundfläche vor, bis sie mit den Zellen der anderen Seite Fühlung gewonnen haben". The process continues until "eine mehrfache Schicht die Wunde bedeckt" (p. 417). Among invertebrates, Rand ('04, p. 39), in his study of regeneration in the earthworm, states that "the wound surface becomes completely covered by an epidermal layer derived from the existing epidermis, without the occurrence of cell proliferation in that layer".

V. The Formation of Segments and Joints.

In this section will be described the earlier stages in the regeneration of the limb. The description of the further differentiation of the tissues and the sequence in which the segments develop is deferred to a later section.

At the time when the first mitotic cell division appears there is already present a thin lamella of chitinogenous cuticle formed on the outer surface of the epidermal cells (Fig. 9, ch^1). The fact that a cuticle could be detected as early as the twenty-fourth hour after operation indicates that cuticle differentiation may begin before cell division has occurred.

With the appearance of mitosis, there soon follows a rapid accumulation of cells in the central region of the epidermal disc of cells which has formed over the injured surface of the stump. This disc of ectodermal cells consequently becomes thicker, its cuticle increases in amount, and at the end of fifty hours the regenerating cells (Fig. 9) are seen pushing outward and breaking through the blood clot at the exterior, to form a papilla-like mass near the center of the stump.

Examined in section, the papilla is seen to consist of an evagination of regenerating epidermal cells. Toward the end of the third day after the operation, the regenerating bud has increased considerably in size (Fig. 10). A characteristic developmental phenomenon is that the lateral or outer wall of the bud grows faster than the inner or more mesial wall, so that, of the two sides, the outer wall becomes both thicker and larger in extent of surface, and contains a greater number of mitotic figures and nuclei. It is also on the outer or ventral sides that the first appearance of joint formation becomes apparent (Fig. 31). The result of this asymmetrical growth is that the regenerating bud bends upward or inward toward the body of the animal; a position which evidently reduces the chances for injury through friction with external objects.

It is near the end of the third day, too, that the first differentiation of limb segments becomes evident. A slight groove appears near the apex of the bud, and thus marks the first step in the development of the two jaws of the claw. It is characteristic that this groove is not located directly at the apex of the bud, but is somewhat ventral to the tip, with the result that during the earlier stages of development the dactyl is relatively larger than the opposing segment or index of the claw. The fact that this relation in the size of the dactyl and the index becomes reversed in the fully regenerated claw, is especially interesting since it is a process parallel with the series of changes which occur in the normal ontogeny of the claw (Emmel '06). During the next six days the outlines of the dactylopodite and propodite become more definite; the anlagen of all the segments appear, and eventually the four joints of the regenerating limb become clearly defined (Fig. 31-36).

As each point develops, an ectodermal invagination arises in relation with it. This invagination, which more or less completely surrounds the joint, soon develops, at two opposite sides of the joint, into two proximally directed processes, which are destined to form attachments for the flexor and extensor muscles. In the interior of each invagination, there is secreted a lamella of chitin which is readily demonstrated by its differential stain with Congo red.

During the development of the joints the epidermal cells present

characteristic structural changes. The mitotic figures occur at the periphery of the bud, the spindle being almost invariably parallel with the surface. A dividing nucleus is rarely found at a deeper level. The cytoplasm is granular in appearance. No cell walls were detected. In the vicinity of the mitotic figure, however, the mass of cytoplasm immediately surrounding the dividing nucleus is less granular and takes on a lighter stain.

Fig. 15 (five days and ten hours after operation) represents a group of epidermal cells in the region of the invagination for the flexor muscle of the fourth segment. The four nuclei at the right in this figure are migrating inward in the process of invagination. The three nuclei at the left are in the epidermal wall of the segment, while the remaining nucleus, in the prophase (?) of mitosis, occupies the typical superficial position. In these nuclei there may again be seen a polarization of the nuclear material similar to that described in the earlier stages of the regenerating cells. The dark staining chromatic elements are found at the inner or more mesial pole of the nucleus (with the exception of the nucleus undergoing mitosis). At the same time the opposite pole of the nucleus takes only a light stain and evidently contains a much smaller amount of chromatin. Here again the position of the plane of division between these light and dark zones varies with the position of the nucleus. (In Fig. 15 the left and right parts are respectively distal and proximal with reference to the limb.) The conditions generally found may be stated as follows: In nuclei lying approximately transverse to the long axis of the regenerating bud, the darker staining material is at the inner end or pole of the nucleus. In nuclei not lying in a transverse plane, as occurs in the region of joint formation, the chromatic material, although still found in the inner region of the nucleus, now becomes shifted either to the proximal or to the distal side of the nucleus according as the inner pole of the nucleus is directed in a distal or a proximal direction. The darker pole also frequently contains a darker body or nucleolus, while at the lighter pole of the nucleus there was usually found a reticular arrangement of fine granular elements. The inner or darker pole of the nucleus was generally found smaller and more tapering or pointed in form as

compared with the more expanded outer peripheral region. Here again the significance of this apparent polarization of the nuclear contents is not clear, but, as was mentioned in the case of migrating nuclei, the general occurrence of these structural characters in different specimens and with different stains, and the variation of the polarization of the elements in conformity with variations in the position of the nuclei, hardly permits of the interpretation of this phenomenon as a mere artifact.

As the invagination of the ectodermal cells at the joints advances, the involved nuclei undergo further typical changes. Fig. 17 (seven days, six hours) represents several nuclei taken at a later stage in the development of the same invagination shown in Fig. 15 (five days, ten hours). A marked difference is at once apparent in these two groups of nuclei. In Fig. 17 the chromatin is evenly distributed, and the nuclei have become elongated to such a degree that frequently they are more than two and one-half times longer than at an earlier stage. See Fig. 16 (six days, six hours) for intermediate stages. It will also be observed in this latter figure that at the center of the invaginating mass of cells, a thin lamella of chitin (*mp*) is now becoming evident, which later serves for muscle attachment.

VI. Origin of New Cells.

We have already learned that the regenerative process is initiated by epidermal cells, which migrate across the wound, form a layer over the injured surface, then multiply by mitotic division and evaginate to form the new bud. It is evident, therefore, that the outer layer of cells covering the regenerating limb bud is entirely of ectodermal origin.

The evagination of the ectodermal cells encloses a cavity at the center of the bud, which becomes filled with a core of cells. The origin of this core of cells becomes at once a vital question in the further study of the histogenesis. Since the interior of the fully developed limb consists of such tissues as striated muscle and connective tissue, it might be expected that this internal core of cells would be derived from similar tissues in the old stump, and consequently would be mesodermal in origin.

A careful study of the successive stages of the regenerating bud, however, does not warrant such a conclusion. Fig. 9 is typical of the conditions found at early stages of the regenerative process. The section is taken through the large limb nerve (n^2) which is here seen passing through a mass of connective tissue (ct). The regenerating layer of ectodermal cells (ec) is increasing in thickness. Between it and the connective tissue membrane enclosing the nerve trunk, a small space has arisen in which may be observed several nuclei lying in what seems to be a syncytial mass of cytoplasm. In their form, structure, and reaction to stains, these nuclei resemble the regenerating ectodermal nuclei. At certain points they are evidently migrating directly from the ectodermal layer of cells; and occasionally nuclei are seen which seem to be migrating inward from the edges of the old epidermis. On the other hand no evidence was obtained indicating any proliferation of the old connective tissue cells. Mitotic figures were not found, nor did there seem to be any migration of the connective tissue cells into the space beneath the evaginating ectodermal plate. Occasional blood cells, however, were observed within this space.

Fig. 10 (two days, twenty-two hours) is a section of a regenerating bud in which the invagination for the two jaws of the claw has begun. The central core of cells is now considerably larger. A few blood corpuscles are present in the proximal region of the core, but the nuclei of the central mass of cells still resemble the ectodermal nuclei. A slight migration of ectodermal cells may be observed from the sides of the limb bud, but it is in the region of the invagination for the first joint that the migration is most extensive. From this advancing invagination the ectodermal cells migrate in large numbers, and thus fill the central cavity of the regenerating bud. In this central mass of cells mitotic figures were very rarely observed. As other limb joints are formed, a similar migration of ectodermal cells was found to occur at the invagination for each limb segment. No evidence was found indicating the possible derivation of these internal cells from the underlying connective tissue of the old stump; if it does occur, it seems evident that it cannot be to any large degree. This conclusion is based upon (1) the

lack of evidence of cell division in the connective tissue, and (2) the fact that at no stage of development did there appear to be a migration of connective tissue cells into the regenerating bud.

In a word, the results of these observations lead to the unexpected conclusion that certainly the greater part, and probably the entire mass, of cells composing the interior as well as the wall of the regenerating limb bud, are ectodermal in origin.

VII. The Differentiation of Tissues.

1. *Striated Muscle*—a. *Histogenesis.*—The first differentiation of striated muscle, as indicated by the formation of myofibrillæ, became apparent as early as five days and twenty-two hours after amputation. On the sixth day the fibrillæ were readily distinguished. At the center of a longitudinal section through the second segment or propodite (Fig. 16, six days, six hours) may be seen the tongue of ectodermal cells invaginated for the flexor muscle of the dactyl. The space between the epidermal cells (*e*) of the regenerating exoskeleton, seen at the lower side of the figure, and the invagination, is filled by a mass of cells, the ectodermal origin of which has already been considered. It is within this central mass of cells that the myofibrillæ (*mf*) first appear. These fibrillæ, instead of extending as straight fibers between their origin in the invagination and their insertion in the epidermal wall, are considerably curved, being convex in a proximal direction. This curvature of the myofibrils does not entirely disappear until after the moult of the lobster has occurred and the regenerated limb has become functional. It may be justly questioned whether there is any correlation between the appearance of these fibrils and functional activity, such, for example, as Eycleshymer ('05) describes in the Necturus embryo, for there is no evidence of functional activity in the regenerated limb until it has been liberated from its enveloping membrane during moulting.

The cytoplasm in which the myofibrils are first seen to differentiate (Fig. 22) appears finely granular in structure and is traversed by a delicate cytoplasmic reticulum (*rt*). As soon as the fibrillæ can be distinguished from the cyto-reticulum they appear as heavier fibers

(*mf*) taking a darker stain with Congo red. The very earliest fibrils are, however, not so readily identified on account of their similarity to the cytoplasmic network and their close relations with it; for at the time of their first differeniation the fibrillæ stain but slightly with Congo red, are irregular or wavy in structure, and appear to be in continuity with the cyto-reticulum. These characteristics indicate that the contractile elements in the regenerating limb of the lobster may be derived from the cyto-reticulum, and consequently favor the "network" rather than the "fibrillæ" theory for the origin of striated muscle fibers. It should be added, however, that no such constancy in relation was observed between the cytoplasmic network and the fibrillæ, as would seem to be necessitated by MacCallum's ('98) theory for striated muscle of vertebrates.

As late as the seventh day of regeneration each myofibril (Fig. 23) still retains its individuality as a single structure. During the eighth day, the fibrils began to appear double or in pairs. In Fig. 24 one of the fibrils is still a single structure, but the remaining eight fibrils are in pairs, the members of each pair appearing in cross section as half cylinders. At later stages of differentiation each pair of fibrillæ becomes represented by a group of four fibrils; the number in each group then increases until as many as twenty fibrillæ could be counted in each bundle, which in transverse section may now be recognized as a "Cohnheim's area". As to how the fibrillæ multiply, the evidence from appearances in both cross and longitudinal sections of the regenerating muscle in this invertebrate supports the conclusions of Heidenhain, Eycleshymer, and other investigators for the striated muscle of vertebrates,—that an increase in the number of myofibrillæ arises through longitudinal division of the fibril.

Preceding the first division or splitting there is a marked increase in the diameter of each fibril (compare Figs. 22 and 23). At the same time the cytoplasm immediately surrounding the fibril becomes less granular and is distinguished from the neighboring cytoplasm by its lighter stain (Figs. 23 and 24). The developing fibril is consequently enclosed by a sheath of modified cytoplasm, which is evidently correctly interpreted as representing the beginning of a Cohnheim area of the mature muscle fiber.

Reference has just been made to the fact that each myofibril retains its individuality as a single structure until the seventh day of regeneration. At this time the light and dark bands of the myofibrillæ are already present, but the "Z" line, or membrane of Krause, could not be observed. By the eighth day the "Z" line had become differentiated, the first indication of these lines being found during the early part of the seventh day. It appears, therefore, that the "Z" lines differentiate later than the light and dark bands. In this respect the differentiation of the regenerating crustacean muscle resembles the histogenesis of the striated muscle as found in the 21 mm. pig embryo, where, as described by Bardeen ('00, p. 392), "The fibril bundles are composed of longitudinal fibrils made up of longer deeply staining segments alternating with shorter lighter segments which do not stain. It is only in older embryos that lines through the light areas, corresponding to Krause's membrane, may be clearly distinguished."

The problem of the origin of the multinucleated muscle cell and its sarcolemma in the regenerating tissue of the lobster differs from the same problem in the vertebrates, because in the former there apparently are no myoblasts concerned in muscle formation.

The structure in the adult muscle of the lobster, which is known as the sarcolemma, is a membrane surrounding the muscle fiber and containing elongated nuclei. This membrane is usually regarded as developed ontogenetically from connective tissue elements. No evidence, however, was obtained for a similar origin during regeneration. When the myofibrillæ first appear in regeneration, definite cell membranes cannot be identified, but the sarcolemma first appears in a later stage of differentiation (Fig. 25, *sa*), and seems to arise by a modification of the cytoplasm of the ectodermal cells.

During the differentiation of the myofibrillæ (which are at first placed centrally and later become located eccentrically in the muscle fiber), the nuclei undergo certain characteristic changes in form and structure. The elongation of the ectodermal nuclei, as they proliferate and migrate inward at the joint, has already been described. As the migration advances, the nuclei in the region where the myofibrils differentiate, assume a peripheral position in the developing

muscle fiber and become more spherical in form (Figs. 16, 22, 24, and 25). These nuclei now seem somewhat larger than the earlier ectodermal nuclei, an appearance which may be partly due to the change from an elongated to a rounded shape. The chromatin appears coarsely granular and has a fairly even distribution, the nucleus as a whole taking a lighter stain. In later differentiation the nuclei again become flattened and considerably elongated in the direction of the long axis of the muscle fiber. The present observation indicates that the more peripheral of these nuclei eventually become the nuclei of the sarcolemma, but more evidence is necessary to establish this point.

In the formation of muscle fibers from a syncytium of ectodermal cells, each fiber is from the very first multinucleated. Instead of "each muscle bundle developing from a single cell" (Claus, '86, p. 33), as seems to be the case in the normal development in Branchipus, for example, each bundle is multicellular in origin. Furthermore, the nuclei increase in number, with the growth of the fiber, by mitotic division, the spindle frequently occurring at right angles to the long axis of the fiber. Consequently in regard to the discussed question of direct and indirect division of muscle nuclei, it is evident that in the regenerating muscle of the lobster at least, mitotic division persists even after the differentiation of myofibrillæ has advanced to a considerable degree.

The conclusion that regenerating striated muscle is ectodermal in origin, draws attention to the problem of the genetic relationship of tissues and the primary germ layers, the question at issue being whether in regeneration a given tissue arises from the same germ layer as in normal development. An adequate discussion of this question in the case of the crustacean muscle, involves, of course, an accurate knowledge of the histogenesis of the tissue under consideration, in both the regenerative and ontogenetic processes.

With regard to regeneration, as a result of the work of Reed ('04) on the crayfish, Ost ('06) on Oniscus, and the present study of the lobster, we have at hand a fairly exact mass of data concerning the genesis of the regenerating crustacean muscle, but as for its origin during normal ontogeny, the evidence does not appear suffi-

ciently conclusive. In the ontogeny of vertebrates, the striated musculature is evidently mesodermal in origin, but whether the same is true in crustacean development, especially in the case of the limb musculature, is not, so far as the writer is aware, clearly established. Consequently it seems obvious that we are not as yet justified in concluding that the ectodermal origin of the regenerating crustacean muscle is a divergence from ontogenetic development. Indeed, as Ost ('06, p. 312) points out, it is not impossible "dass die embryonalen Muskeln von den ectodermalen Sehneneinstülpungen aussprossten und dann Regeneration und Embryonalentwicklung sogar übereinstimmten," a conclusion which is certainly not rendered less improbable when one considers the degree to which the generally accepted conclusions regarding genetic relationships between ectoderm, mesoderm, and certain forms of connective and muscle tissue have been called into question by the work of such investigators as Katschenko ('88), Kölliker ('84), and Platt ('98).

b. *Attachment to the Exoskeleton.*—Among the first investigations on the attachment of the crustacean muscle is Claus's ('86) work on Branchipus and Artemia. Claus concludes that in many cases the muscle fibers are attached directly to the exoskeleton (pp. 22 and 29), a conclusion which involves the assumption that among some invertebrates at least the muscle may be attached to the skeleton without the intervention of a connective tissue tendon, such as are typical of muscle-skeletal attachments among vertebrates. Since 1886 the subject has received considerable attention both for crustacea and insects, with the result that several divergent opinions have arisen. As far as the writer is aware, however, the problem has not been studied from the standpoint of regeneration; consequently data derived from the present investigation of the lobster may not be valueless.

Attention has already been directed to the fact that in the early stages of the regenerating bud and up to the time when the muscle fibrils have become well differentiated, there is no definite boundary between the outer epithelial cells related to the chitin and the internal cell mass in which muscle develops. Cell walls are not evident, and the cytoplasm of the two regions appears syncytially related, the

only noticeable difference being that the epidermal cytoplasm next to the chitin takes a slightly darker stain (Fig. 16). The question now arises, what is the relation of the developing myofibrillæ to the external epidermal cells and how is their attachment to the chitin established?

In studying the regenerating tissue at the stage when the myofibrillæ can just be identified (Fig. 16, six days, six hours), an important fact becomes evident, viz., that fibrils develop in the epidermal layer of cells, simultaneously with the differentiation of the myofibrillæ in the internal cells, apparently without any discontinuity between them. In other words in early differentiation the myofibrillæ can be traced into the layer of epidermal cells, and in some cases at even this early stage (six days, six hours) myofibrils were found continuous even through to the chitin.

The differentiating muscle fibrils early assume a characteristic striation (Fig. 18, *mf*). At the same time more clearly defined differences are becoming evident between the epidermal and muscle cells. The muscle nuclei are more nearly spherical in form, while the epidermal cells take a distinctly heavier stain. Small vacuoles can also be observed at the boundary between the two groups of cells. The striation of the muscle fibrils gradually becomes more distinct. These striations could be traced beneath the inner surface of the epidermis, frequently extending to the level of the proximal ends of the nuclei (Fig. 18). No evidence was obtained, however, that the fibrillæ are ever striated entirely through the epidermis to the chitin.

A definite boundary (basement membrane) now becomes apparent between the epidermal and muscle cells (Fig. 19, *em*). There is an interesting variation in the general level of the boundary or inner surface of the epidermis in the region of muscle attachment. The epidermis between the attachment of two or more muscles, frequently projects inward in rounded elevations or broad papillæ. The result is that in the immediate vicinity of the muscle attachment the inner surface of the epidermis appears to follow the fiber for some distance toward the chitin, thus forming a sort of pit, through the bottom of which passes the muscle fiber (Figs. 19 and 20). Since this elevation of the epidermis increases in the later stages of the regenerating

limb, it is probably due to the rapid growth of the epidermal cells, which are consequently forced inward between the muscle fibers. After the moult, the regenerating limb expands and the elevations then disappear as the epidermis spreads out to cover the now larger surface of the limb.

The muscle fibers appear to be continuous throughout the epidermis to the chitin (Figs. 18, 19, and 20). Frequently just before the fibers reach the chitin they spread out in a brush-like manner and fuse with the chitin (Fig. 20). In this fusion the fibrils frequently terminate on knob-like thickenings or inward projections of the chitin (Fig. 19). These chitinogenous processes were found only in connection with the attachment of the regenerating muscle fibers.

In later stages of differentiation, the epidermal nuclei adjacent to the muscle attachment frequently become considerably lengthened in the direction of the long axis of the muscle fiber. In addition to the fibrils concerned with muscle attachment, there are other fibrils which later differentiate in the cytoplasm of the epidermal cells (Fig. 19, *ef*). These latter fibrils do not appear to be associated with muscle attachment. In structure they are much finer and are distinguished from muscle fibrils by their stain reaction. With Mallory's connective tissue stain they appear light blue, whereas the fibrils concerned with muscle attachment take a dark red or purple stain similar to the striated part of the myofibril.

On the inner surface of the fully developed epidermis of the crustacean there occurs a very thin layer or lamella of tissue which has been termed the "Grenzlamelle" (Schneider). The fact that a flat nucleus is occasionally found in this "border lamella" has favored the interpretation that it is a connective tissue formation and consequently mesodermal in origin. Other investigators (Claus, for example) have regarded this layer or "basement membrane," as merely a differentiation of the inner surface of the epidermal cells. In regard to this question it is clear that in the early regeneration of the lobster's limb there is no definite limiting membrane between the epidermal and muscle cells. The first limiting membrane to appear during the differentiation of the epidermal cells is evidently

epidermal in origin and to that extent is ectodermal. This earlier membrane seems similar to the basement membrane which McMurrich ('96) describes on the epithelium of the mid gut of isopods, and which he concludes "was formed from the epithelial cells and not by the mesoderm" (p. 89). It is to be observed, however, that at a later stage of development (Fig. 21) a thin nucleated layer does appear on the inner surface of the epidermis, which is perhaps to be regarded as the true "Grenzlamelle." The origin of this latter layer has not been satisfactorily determined. It was noticed that it is first apparent in the region of the developing blood sinuses, where it appears to serve as a vascular epithelium.

c. *Discussion and Conclusions.*—The study of the attachment of the arthropod muscle is attended with considerable technical difficulty. That the question is still an open one is indicated by the diverse opinions existing at the present time. The chief points at issue are involved in the solution of the questions whether the muscle fibers are attached (1) directly to the chitin of the exoskeleton, or (2) indirectly by means of intervening epidermal cells; and in the latter case, whether (3) in addition to the epidermal cells there is also an intervention of connective tissue between the muscle fibers and the epidermis, in a manner somewhat analogous to the muscle attachment typical among vertebrates.

The literature upon this subject is becoming very extensive, especially for insects, where the question of muscle attachment has been studied by Henneguy ('06), Holmgren ('02), Lecaillon ('07), Riley ('08), Snethlage ('05),[5] and others. Without reviewing this growing literature, we may advantageously confine our discussion to the present observations on the lobster and their bearing on the problem among crustacea.

The third of the different modes of muscle attachment just considered involves the union of three sets of fibrils,—the fibrils of the muscle, of connective tissue, and of the epidermal cells. Schneider ('02) is inclined to regard this method of attachment as typical for crustacea, and his description of the jaw muscle of the crayfish states

[5]Unfortunately, I have been able to have access only to abstracts of Snethlage's work, "Ueber die Frage vom Muskelansatze, etc., bei den Arthropoden."

that "Das Bindegewebe ist an den Muskelenden als typisches Fasergewebe entwickelt, das sich vom faserigen Zellengewebe durch reichliche Entwicklung extracellulärer fibrillärer Bindesubstanz, im Umkreis spindeliger Bindezellen scharf unterscheidet. Die Myofibrillen einerseits und die Deckzellenfibrillen andererseits senken sich in eine dicke Lamelle ein, in der Bindefibrillen in dichter Anordung, von spärlicher Grundsubstanz verkittet, entsprechend den Myo- und Stützfibrillen verlaufen" (p. 494). On the other hand, Claus ('86) in his study of Branchipus finds that "An vielen Stellen heften sich aber die Muskelsehnen nicht direct mittelst Connectivfäden am Integument an, sondern gehen in einer durch solche suspendirte Lamelle über, welche sich der Integumentfläche parallel als Basalplatte unterhalb jener ausbreitet" (p. 29).

The critical question here is in regard to the continuity of the muscle fibers within the epidermal cells. If the muscle fibers can be traced below the level of the inner surface of the epidermis, independent of any connective tissue elements, evidently the third mode of attachment may be eliminated from further consideration. That such is certainly the case in the regenerating musculature of the lobster's limb has already been indicated, and the crucial fact remains that this relation of the myofibrillæ was already evident at a stage of development before there was any differentiation of connective tissue elements or of a "Grenzlamelle." Consequently, at this time at least, the muscle fibrils are found passing into the epidermis unaccompanied by any connective tissue elements. The apparently close relation, which is later established between the muscle fibers and the connective tissue, may perhaps be not incorrectly regarded as a secondary development resulting from a later differentiation of connective tissue around the already formed muscle fibrils, rather than as a primary functional relation. It is of interest to observe that even in the fully developed lobster, as admitted by Dahlgren and Kepner ('08), the connective tissue elements are in some places "so small as to be apparently absent, and it would seem possible that in some attachments they were absent altogether and the muscle joined directly with the epithelium" (p. 66).

In considering the two remaining modes of attachment, certain

aspects of the problem, as they present themselves in other animal forms, disappear in approaching the subject from the standpoint of regeneration. For example, the question whether the fibrils by which muscle attachment is accomplished are inter- or intra-cellular with reference to the epidermal cells, loses much of its significance here on account of the syncytial structure of the cytoplasm. Genetically, there is perhaps no distinction to be drawn between the purely epidermal fibrils and the myofibrillæ, for if the conclusions regarding the origin of the muscle are correct, both groups of fibrils are derivatives of the cytoplasm of epidermal cells, and are consequently also both ectodermal. Structurally, however, they have diverged in their differentiation, in conformity with their differences in function. The striation of the muscle fibril can frequently be traced for some distance into the epidermal cytoplasm, but it was not observed that the striation of the fibrils ever continues through to the chitin; it appears that the peripheral ends of the original myofibrillæ diffentiate as tensile rather than as contractile structures. But even the non-contractile elements thus directly concerned with the attachment of the muscle to the chitin can be distinguished from the purely supportive fibrils of the epidermal cells by (a) their staining reaction with Congo red and Mallory's stain; (b) their frequently larger size; (c) their evident continuity with the contractile part of the muscle fiber; and (d) their relations with the exoskeleton where they terminate in characteristic end-plates or chitinogenous processes.

The conclusions which these observations support may, therefore, be summarized as follows:

1. The myofibrillæ differentiate in the cytoplasm of a syncytial mass of cells, derived from the epidermis and consequently ectodermal in origin.

2. The original fibrillæ ultimately differentiate throughout their whole length into true striated muscle elements, except in the region of skeletal attachment, where the peripheral ends of the fibrils remain unstriated, and serve as tensile structures directly uniting the contractile elements with the chitin of the exoskeleton.

3. In later development connective tissue elements may form over the inner surface of the epidermis and around the muscle fibers, and

purely supportive fibrils differentiate within the epidermal cells, but these structures are secondary in their relation to muscle attachment.

2. *Nerve Fibers.*—In studying the regeneration of nerve fibers we are at once involved in the intricate questions of the origin of the neurilemma, and of the axis cylinder with its neurofibrillæ. Complete agreement regarding ectodermal or mesodermal origin of these structures has not yet been attained. Regarding the neurilemma, for example, the conclusions of one group of investigators may be summarized in the statement that sheath cells "are true connective tissue cells from the locality through which the nerve fiber has passed in its development" (Dahlgren and Kepner, '08, p. 188). On the other hand, the ectodermal origin of the sheath cells appears to be strongly supported by experiments in which it has been shown that in the tadpole at least "the source of the sheath cells, both of the motor and sensory nerves is in the ganglion crest" (Harrison, '08, p. 393). Although the present observations on the lobster are, on account of a lack of material especially prepared for neurological study, not sufficiently extensive to render them very important in relation to these fundamental problems, still they may be of some value.

In early stages of the regenerating limb bud there is found a cord or column of cells extending from the tip of the bud to the end of the old nerve trunk. As the limb segments are formed, this cord of cells is joined by similar strands from the various segments. Within these cords the axis cylinders of the nerve fibers develop, and from the cells composing the cord the sheath cells are differentiated. The important question is as to the origin of these cords of cells; have they migrated outward from the sheath cells of the old nerve trunk, or are they derived from the ectodermal cells of the regenerating bud?

The present observations support the latter conclusion. Among the old sheath cells no evidence of cell division was obtained, nor was there observed any extensive proliferation or outward migration of the sheath cells from the old nerve trunk. On the other hand, cells from the first-formed ectodermal plate migrate inward toward the injured nerve, as has already been described in the early

stages of the regenerating bud (Fig. 9). At later stages this inward migration of ectodermal cells is quite marked in certain regions (Fig. 28, *n*), where they evidently contribute to the formation of the cords of cells just described. The distal end of each cord always contains a larger number of cells than its more proximal end, giving it a club shape, with the smaller end joined to the old nerve. Occasionally mitotic figures were found, but they always occurred among the more distal cells of the cords. The nuclei of these distal cells were generally large and spherical in form, but in passing proximally toward the old nerve trunk, there was a gradual transition from the spherical nuclei to the long flat nuclei of the sheath cells, indicating a direct differentiation of the neurilemma from these ectodermal cells.

These observations on the lobster, together with the results of Reed's ('04) on the crayfish, and Steele's ('07) on the nerve endings in the ommatidia of the eye, furnish collective evidence for the participation of ectodermal cells in the regeneration of the nerve fibers in crustacea. It remains to be determined, however, whether this conclusion can be extended to arthropods in general. For it must not be overlooked that different results have been obtained by Ost ('06) in his study of the regenerating antenna of Oniscus, in which form he finds that the neurilemma regenerates "durch Nachschieben vom proximalen Ende her . . . Die Regeneration des Antennennerven von Oniscus geht also nach meinen Beobachtungen durch direktes Auswachsen junger Nervenfasern aus dem alten Stumpf vor sich" (p. 313). It is noteworthy that Ost was unable to obtain any evidence of cell division among the sheath cells, for he states, "Mitosen oder sonstige Teilungsvorgänge konnte ich an diesen Kernen freilich nie beobachten,"—a result corresponding with the conditions found in the regenerating lobster's limb, *i. e.*, in the region of the old nerve trunk.

By the fifth day of regeneration (five days, ten hours) the differentiation of an axis cylinder within the cord of cells just described, had advanced sufficiently to present delicate neurofibrillæ.[6] These

[6]The nerve fiber thus appears to develop earlier than the muscle, for in all cases regenerating nerve fibers were found before myofibrillæ could be detected.

fibrillæ were not of equal size and were distributed in an interesting manner. In some of the preparations, the larger or heavier fibrils were mostly at the periphery of the axis cylinder, whereas the finer ones were nearer the center (Fig. 29). Consequently at certain stages of regeneration the nerve fiber could be structurally analyzed into four parts: (a) a central core of very delicate fibrillæ; (b) a peripheral layer, not sharply marked off from (a) but containing heavier fibrils; (c) the external sheath of nucleated cells or future neurilemmma, and (d) a sheath of cytoplasm between (b) and (c) relatively free from neurofibrillæ. In later stages of differentiation the heavier fibrils predominate (Fig. 30). At the time when the neurofibrillæ are beginning to appear, the cytoplasm of the nucleated sheath, as compared with that of the axis cylinder, is more coarsely granular. In some regions there also appears to be a membrane present between the nucleated sheath and axis cylinder.

No conclusive evidence was obtained as to whether the axis cylinder is formed in situ from the sheath cells, or whether it is an outgrowth from the axis cylinder of the old nerve cell. In either case, however, if the present observation is correct, that the nerve fiber develops within a cord of cells proliferated from the ectoderm of the various new limb segments, it points to the sheath cells as one of the factors involved in determining the final distribution of nerve fibers within the regenerated limb. It was not determined whether the neurofibrillæ differentiate distally or proximally, or whether they appear simultaneously throughout the regenerating nerve fiber. The fact that in at least certain stages of differentiation the finer fibrillæ occur at he center, and the heavier fibrils at the periphery of the fiber, apparently lends support to the conclusion that the neurofibrillæ are being derived from the cytoplasm of the axis cylinder.

3. *Connective Tissue.*—The connective or supporting tissue of the crustacean limb consists mostly of broad loose bands or sheets extending between opposite walls of the exoskeleton, around the extremities of each segment, and in certain regions filling almost the entire segment. In addition to these sheets and bands of connective tissue there is also a thin layer of simple flat epithelium found covering the inner surface of the epidermal cells (here known as "Grenzlamelle") and surrounding the muscle bundles.

Let us consider first the broad sheets of tissue extending from one wall to the other within the limb segment. As the segments of the regenerating limb become well differentiated, there occurs an extensive inward migration of ectodermal cells in those regions where there later develop the bands of connective tissue characteristic of the adult limb. This migration is especially well defined in the regenerating meropodite. The epidermal cells in this region migrate inward from the epidermal wall and form a broad sheet extending across the central cavity of the segment (Fig. 26, *ic*). The nuclei become elongated, and distinct fibrils arise in the cytoplasm. At later stages (Fig. 27, eleven days, ten hours) the cytoplasm, with the exception of the epidermal cells next the exoskeleton, becomes vacuolated. These intercellular spaces then become filled with blood plasm (Fig. 27, *b*), and eventually there is thus formed the vascular and connective tissue characteristic of the fully developed limb.

It seems evident, therefore, that in at least certain regions of the regenerating limb a supportive and apparently true connective tissue may be derived from ectodermal cells. Nor is this divergent from conditions found in normal development among crustacea, for Claus ('86) in his work on the embryology of Branchipus, concludes that the broad connective tissue bands which unite the opposite surfaces of the integument, "sind Erzeugnisse der Chitinogenzellen der Hypodermis", and quotes Braun to the effect that in the crayfish it is "nicht möglich, eine scharfe Grenze zwischen den Erzeugnissen von Chitinogenzellen und den mesodermalen Bindegewebebildungen festzustellen" (pp. 22-23).

We may now briefly consider the tissue characteristics of the so-called "Grenzlamelle." In the description of the regenerating muscle, reference has been made to the fact that a "Grenzlamelle" is not present under the epidermis at the time when myofibrillæ begin to differentiate. It is only after the muscle fibers and the epidermis have become well developed that an epithelial layer is formed over the inner surface of the epidermal cells. In various parts of the limb this "Grenzlamelle" is apparently the only tissue between the blood sinuses and the epidermis, and consequently in at least certain regions it seems to serve as a vascular epithelium (cf. Williams, '07, p. 155).

As to the origin of the regenerating "Grenzlamelle," the present observations fail to furnish conclusive evidence. The fact, however, that the regenerating bud becomes filled with ectodermal cells involved in the regeneration of muscles and nerves, and the lack of clear evidence of cell proliferation from the old connective tissue, suggests an ectodermal origin. Of interest in this connection are Claus's observations on the normal development of Branchipus:— "Was man als solche (connective tissue) beim ersten Blick in Anspruch zu nehmen geneigt ist, erweist sich wenigstens an vielen Stellen nach genauer Betrachtung als eine Art innere Cuticula, die aus der Basis des Epithels durch Erhärtung des Protoplasmus entstanden ist" (p. 21). On the other hand, Schneider ('02) from a comparative point of view is unable to support this conclusion: "Nach Claus stammen die Grenzlamellen und die Muskelsehnen mindestens zum Teil vom Epiderm, dessen Deckzellen die basalen ausscheiden sollen. Dieser Ansicht kann in Rücksicht auf die Verhältnisse bei anderen Arthropoden, wo die Sehnen und Lamellen unzweifelhaft Bindegewebsbildungen sind, nicht beigestimmt werden, wenn auch die Bildung von Seiten des Bindegewebes nicht bekannt ist" (p. 466).

VIII. The Direction of Differentiation.

The question of the direction in which the process of differentiation proceeds in both regeneration and normal ontogeny is a subject which has given rise to diverse opinions. In elaborating certain theories of form regulation, Holmes ('04) assumed that in a regenerating appendage, differentiation proceeds from the base toward the tip, whereas Child, at least in an earlier paper ('06), was inclined to regard the reverse as generally true.[7] Zeleny ('07) found in the regenerating antennule of Mancasellus that there are two distinct periods of development, in the first of which the process of differentiation "begins at the base and travels irregularly outward", while in the second period, the process is in the reverse order, *i. e.*, beginning with the terminal segment and differentiating inwards (p.

[7]Holmes ('07) and Child ('08) have later restated their theories as not being inconsistent with differentiation in either direction.

325). Haseman ('07) concluded that in the regenerating cheliped of the crayfish, differentiation proceeded in a disto-proximal direction. Haseman, appreciating the importance of determining whether changes in external form could be safely taken as an index of internal changes, gave some attention to the internal differentiation of tissues. But as a rule conclusions upon this subject have been largely based upon evidence derived from modifications in external structure, rather than from internal cytological and histological changes in the segment tissues. In view of this fact, the following study has been made with the purpose of obtaining further data upon the direction of differentiation among the internal regenerating tissues.

The results to be described were derived from the study of serial sections of somewhat over a hundred regenerating chelæ at various stages of development. In tracing the progress of differentiation it becomes necessary to rely on such criteria as are most clearly defined. In the present case the following structural characters have been chosen on account of their ready identification by means of selective stains: (1) the formation of chitinogenous muscle plates or tendons; (2) the differentiation of myofibrillæ; and (3) the striation of the muscle fibrils.

In the description of the differentiation of the regenerating limb, it will be recalled that distal to the breaking joint there are five segments. Each segment contains a pair of muscles controlling the extensor and flexor movements of the next distal segment (see Fig. 37). It is important to note that in each pair of muscles the flexor is the larger, and that of the four pairs of muscles, it is the first and third pairs (counting from the distal end) which contain the largest muscles, *i. e.,* the muscles which lie in the propodite and meropodite. Each muscle takes its origin from the exoskeletal wall of the segment in which it lies, and is inserted upon the chitinogenous plate extending proximally from the next distal segment. The differentiation of these chitinogenous plates will first be considered.

1. *The Chitinogenous Plates or Muscle Tendons.*—The differentiation of chitin within the joint invagination is indicated by a characteristic brick-red stain reaction with Congo red, or bright blue with Mallory's connective tissue stain. The developing chitin is thus

sharply contrasted with any surrounding tissue. Using this stain reaction as an index of differentiation, serial sections were studied and graphic reconstructions made of different stages in the regeneration of the limb (Figs. 31-36).

a. *Two Days and Twenty-two Hours after Operation* (*Fig.* 31).—Although externally at this time there was little, if any, indication of segmentation, internally there was found a well defined invagination of epidermal cells, within which a differentiation of chitin (r^1) was already evident. This invagination marks the beginning of the first and second distal segments. It is here that the chitin plate is formed for the flexor muscle of the dactyl, which, it is to be observed, is normally not only much larger than the opposing extensor, but is also the largest muscle of the limb.[8]

b. *Four Days and Six Hours* (*Fig.* 32).—The differentiation of additional chitin plates is not evident until on the fourth day of regeneration. At this time two more invaginations were beginning to develop chitin. Of these two invaginations the one representing the extensor for the dactyl (e^1) showed as yet but a slight differentiation of this tissue. The other invagination (r^3) represents the flexor for the meropodite, and shows a well-defined plate of chitin.

In regard to the latter invagination (r^3) there are two important points to be emphasized. First, the invagination is not in the next or third proximal segment, but in the fourth segment or meropodite; and second, of the two muscles in the meropodite, the invagination represents the flexor.[9]

[8]This invagination occurs at one side of the apex of the bud, so that at first the dactyl is relatively larger than the index,—a relation, it is interesting to note, which corresponds with the condition found in the larval stages of development, but is reversed in the adult. (Emmel, '06[2]).

[9]In the external segmentation of the limb at this stage, the groove between the second and third segments is quite as well marked, if not more so, than the groove between the third and fourth segments. This agrees with Haseman's ('07) observations on the cheliped, and with my own earlier description ('06) for the lobster, that the external segmentation appears to proceed in a disto-proximal direction; but the crucial fact that in the present case it is the tendon in the fourth segment instead of in the third segment which differentiates next after the second segment, demonstrates that the external segmentation cannot, therefore, be safely relied upon alone as a correct index of internal differentiation.

The first point becomes especially interesting when it is considered that according to the requirements of the theory that differentiation proceeds in a disto-proximal direction, the next chitin muscle plate to develop after those of the propodite should have been in the third segment or carpopodite instead of the fourth segment or meropodite. Serial sections of the carpopodite were carefully examined, but there was no evidence that any chitinization had yet taken place for the tendon plates in this segment.

c. *Four Days and Twenty-two Hours* (*Fig.* 33).—At this stage a fourth chitinous tendon (r^2) has become evident. This tendon is in the third segment and is the one involved in the development of the flexor muscle. The extensor (e^1) and flexor (r^1) in the propodite, and the flexor (r^3) in the meropodite, show an advance in differentiation as compared with the preceding stage, but otherwise there is no evidence of chitin differentiation in any of the remaining muscles. Consequently at this stage only four chitinogenous plates or tendons are present; two in the propodite and one in each of the next two proximal segments. The important point is that in each case it is the tendon for the flexor muscle which differentiates first in each segment.

d. *Five Days and Six Hours* (*Fig.* 34).—The tendon for a second extensor muscle (e^3) is now beginning to differentiate. Here again the next tendon to develop is not for the extensor in the carpopodite, but for the one in the meropodite. At this time two other chitinous tendons (e^4) are also just becoming evident in the fifth segment or ischiopodite. The muscles correlated with these last two tendons, it will be recalled, are both extensors.

e. *Five Days and Nine Hours* (*Fig.* 35).—The chitinous tendon (e^2) for the extensor of the carpopodite is now present. This introduces, therefore, the last of the eight muscle tendons to differentiate.

f. *Ten Days and Two Hours* (*Fig.* 36).—By this time the eight chitinogenous tendons and muscles of the regenerating limb have become fully developed. The animal is now about to undergo the process of moulting, in which the regenerated limb will be liberated from its membranous sac and become a functional appendage.

In summarizing these observations it may be stated that the chitin-

ogenous plates or tendons for each of the eight muscles distal of the breaking joint were found to be differentiated in the following order: (1) for the flexor muscle in the second limb segment; (2) the flexor in the third segment, together with the extensor in the second segment; (3) the flexor in the third segment, and at the same time the two extensors in the fifth segment; (4) and last, the extensor in the third segment. From these results it seems evident that the direction of differentiation cannot be described as being any more a disto-proximal one than the reverse. The important point disclosed is that in each segment the tendon for the flexor, or larger muscle of the pair, is differentiated first; and further, of these flexors the larger ones are the first to develop.

2. *Differentiation of Myofibrillæ.*—The results of the microscopical study of the earliest differentiation of the myofibrillæ in each pair of regenerating muscles are presented in the following table. In this table the first column gives the time of regeneration. The signs + and 0 indicate, respectively, the presence or absence of myofibrils.

TABLE SHOWING THE SEQUENCE OF DIFFERENTIATION OF MYOFIBRILLAE IN EACH OF THE EIGHT LIMB MUSCLES DISTAL TO THE BREAKING JOINT.

Series No.	Regeneration.	Second Segment.		Third Segment.		Fourth Segment.		Fifth Segment.	
		Flex.	Exten.	Flex.	Exten.	Flex.	Exten.	Exten.	Exten
29	5 da. 9 hrs.	+	0	0	0	0	0	0	0
30	5 da. 10 hrs.	+	0	0	0	+*	0	0	0
31	5 da. 22 hrs.	+	+	+	+*	+	+*	0	0
33	6 da. 6 hrs.	+	+	+	+	+	+	+	+

*Differentiation just beginning.

In this table it will be seen that the first muscle fibrils to be differentiated were those of the flexor in the second segment; the next to appear were those of the flexor in the fourth segment. Up to this time no muscle fibrillæ were found in either the third segment or in the extensors in the second and fourth segments. The flexors in the fifth segment were the last to develop. Here again, differentia-

tion does not proceed definitely in either a proximal or a distal direction, but appears rather to be correlated with the size and function of the muscles concerned.

3. *Differentiation of Striæ in the Myofibrillæ.*—The series were also studied to ascertain the time at which myofibrils in each muscle showed striations. The following table represents the results obtained. The signs + and 0 indicate, respectively, the presence and absence of striation.

TABLE SHOWING THE SEQUENCE IN THE DIFFERENTIATION OF STRIAE IN THE REGENERATING LIMB MUSCLES.

Series No	Regeneration.	Second Segment.		Third Segment.		Fourth Segment.		Fifth Segment.	
		Flex.	Exten.	Flex.	Exten.	Flex.	Exten.	Exten.	Exten.
34	6 da. 22 hrs.	+*	+	0	0	+	0	0	0
35	7 da. 2 hrs.	+	+	+	+	+	+	+	+†

*Striations were not found until after the myofibrillæ had begun to be differentiated in all the segments.

†Differentiation of striæ just becoming evident.

The time intervening between the appearance of the first striæ and the striation of all the muscles is apparently much shorter than the time between the first differentiation of myofibrils and their complete development for all the muscles. Nevertheless, striation does not occur simultaneously throughout the limb. The striæ differentiate first in the flexor muscles in the second and fourth segments, and later they appear in the remaining limb muscles.

4. *Conclusions.*—The results of the present study of the differentiation of the chitinogenous muscle tendons, the myofibrillæ and their striation justify two conclusions:

First, that the differentiation of the regenerating musculature proceeds in neither a definite disto-proximal direction, nor the reverse.

Second, that whatever sequence there may be in this differentiation is correlated rather with the size and functional relations of the various muscles concerned, the larger muscles differentiating first.

Reference has already been made to the recent discussions of the direction of differentiation by Child ('06, '08), Holmes ('04, '07), Zeleny ('07), and Haseman ('07). Among these investigators Haseman has studied the form most closely related to the lobster. From his observations upon the regenerating appendages of the crayfish, he concludes that differentiation in the chela proceeds from the tip toward the base, while the reverse is true for the walking legs.

In view of the results of these investigators Zeleny has discussed three possibilities regarding the direction of differentiation in the development of an appendage.

1. That "all the parts may appear at once"; (2) that "the progression may be outwardly directed", or (3) "it may be inwardly directed". Zeleny points out that the first lacks evidence, that the second has been more especially favored by Pflüger and Holmes in connection with certain general theories of development, and the third by Morgan, Driesch, and Child. From his own study Zeleny concludes that the second method may predominate in some animals, the third in others, and further that both modes may predominate at different times in the same animal, as appears to be the case in the antennule of Mancasellus macrourus. It is evident that the present data from the lobster fail to conform with any of the three modes of differentiation. But the evidence does support a fourth proposition, viz., that sequence of differentiation may be influenced or determined by the relative size and functional rôle of the regenerating structures. Consequently, differentiation in the regenerating appendage is not necessarily either "centrifugal" nor "centripetal," the new structures, on the contrary, differentiating rather according to size and functional relations, in a manner perhaps similar to the formation of organs in embryonic development,—a most striking illustration of which is furnished by Sutton's ('83) rule for the differentiation of the epiphyses of the long bones in human osteology: "The centers of ossification appear earliest for those epiphyses which bear the largest relative proportion to the shafts of the bones to which they belong" (p. 480).

IX. Summary.

1. Two new structures are recorded in connection with the breaking joint of the chela of lobsters.

a. A muscle crossing the joint, but degenerating during the fifth larval stage.

b. Valves in the venous blood sinus which prevent excessive hemorrhage after the autotomy of the limb. The valves persist throughout life.

The following conclusions concern the regeneration of the limb after autotomy.

2. The first layer of cells, aside from the blood clot, to cover the wound is formed by the migration of epidermal cells.

3. The nuclei of these migrating cells enlarge and become somewhat wedge-shaped. The karyosomes disintegrate into fine granules which tend to collect at the proximal end of the nucleus. Thus the nuclear contents appear polarized into an inner zone of darkly stained chromatin, and an outer zone relatively free from chromatin granules.

4. The significance of this polarization is not clear, but its regular occurrence, and is reversed on opposite sides of the limb, together with the frequent expansion of the higher pole, and the association of axial chromation masses with the darker pole, indicate that it is a phenomenon characteristic of certain stages in the regenerative activity of the epidermal cell.

5. During these nuclear activities the cytoplasm loses its supportive fibrillæ and reticular structure, and becomes finely granular. Although definite cell membranes are never evident, there are more or less clearly defined cytoplasmic units around each nucleus.

6. Mitotic cell division begins after the formation of the first epidermal plate over the wound. The formation of this plate seems sufficiently accounted for by the volumetric increase of the cytoplasm, together with the enlargement and wider separation of the nuclei of the migrating cells.

7. The wall of the regenerating bud, and a large part, if not the entire core of cells filling its interior, are derived from epidermal cells. Since there is no conclusive evidence of either cell division or migration among the old muscle and connective tissue cells, these tissues appear to contribute little, if anything, to the formation of the new limb bud.

8. a. Regenerating striated muscle is ectodermal in origin.

b. The myofibrillæ appear genetically related to the cyto-reticulum. Each fibril is early surrounded by a sheath of modified cytoplasm, multiplies by longitudinal splitting, and in its differentiation the "z" line or membrane of Krause, becomes evident after the formation of the light and dark bands.

c. The muscle fiber or cell is multinuclear when first formed. Later the nuclei come to be peripheral in position while the fibrillæ lie to the side of the fiber nearest the center of the muscle bundle. The nuclei multiply by mitotic division, and with the development of the sarcolemma they become flattened and elongated in the long axis of the fibers.

d. The myofibrillæ differentiate throughout their whole length into true striated muscle elements, except in the region of skeletal attachment. There the peripheral ends of the fibrils remain unstriated, and serve as tensile elements.

e. The connective tissue over the inner surface of the epidermis, and the purely supporting fibrillæ within the epidermal cells develop later, but these structures appear to be secondary in their relation to muscle attachment.

9. a. The present observations indicate that the neurilemma is derived from the regenerating epidermal cells.

b. During certain stages in the differentiation of the axis cylinder the finer neurofibrillæ are central and the coarser fibrils more peripheral in position.

10. In at least certain regions of the regenerating limb, supporting and apparently true connective tissue differentiates from epidermal cells.

11. a. In the development of the chitinogenous muscle plates and the myofibrillæ and their striæ, differentiation appears to be neither directly "centrifugal" nor "centripetal" in direction.

b. On the contrary, the facts warrant the statement that whatever sequence there may be in differentiation, it is correlated with the size and functional relations of the muscles concerned, rather than with any distal or proximal relation of these structures to the organism.

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XI. DESCRIPTION OF PLATES.

Unless otherwise indicated, all figures are from camera drawings made from longitudinal sections of the chelæ of fourth stage lobsters.

List of Abbreviations.

a, artery.

a^1, a^3, arteries passing through the breaking joint.

b, blood plasm.

bc, blood corpuscles.

bcl, blood clot.

bk, breaking joint.

bs, basipodite.

c, carpopodite.

ch, chitin.

ch^1, new chitin.

cm, connective tissue membrane.

ct, connective tissue.

d, dactylopodite.

e, epidermis.

e^1, migrating epidermal cells.

e^1, e^2, e^3, e^4, extensor mucles in the propodite, carpopodite, meropodite, and ischiopodite, respectively.

ec, regenerating and proliferating epidermal cells.

ef, supportive fibrillæ.

em, epidermal membrane.

en, regenerating epidermal nuclei.

i, ischiopodite.

ic, inward proliferation of epidermal cells to form connective tissue.
in, invagination of epidermal cells at the joints
m, muscle crossing the breaking joint.
ma, muscle attachment.
mb, muscle in the basipodite.
mc, meropodite.
mf, myofibrillæ.
mn, muscle nuclei.
mp, chitinogenous muscle plate or tendon.
mt, cells undergoing mitotic division.
n, nerve.
n^1, n^2, nerves passing through the breaking joint.
nf, neurofibrillæ.
ng, nuclei of the "Grenzlamelle."
nn, nuclei of the neurilemma.
p, propodite.
r^1, r^2, r^3, flexor muscles in the propodite, carpopodite, and meropodite, respectively.
rt, cyto-reticulum.
s, septum of connective tissue dividing venous blood sinus into two channels.
sa, sarcolemma.
v, valves in venous blood channels.
v, valves in venous blood channels; v^1, v^2, inner and outer channels, respectively.
vc, venous blood channel.
vs, venous sinus; vs^1, vs^2, respectively distant and proximal of the breaking joint.

PLATE I.

STRUCTURE OF THE CHELA IN THE REGION OF AMPUTATION.

Graphic reconstructions of the breaking joint and related segments of the left chela, to show the anatomical relations of the blood vessels, muscles, nerves, epidermis, and connective tissue. The drawings are incomplete in one particular because the finer meshwork of connective tissue in which the arteries and nerves are suspended is not represented in its entirety.

FIG. 1 represents the outer third, and

FIG. 2 the inner third of the breaking joint and segments. The sides of the figures nearest each other are ventral. × 263.

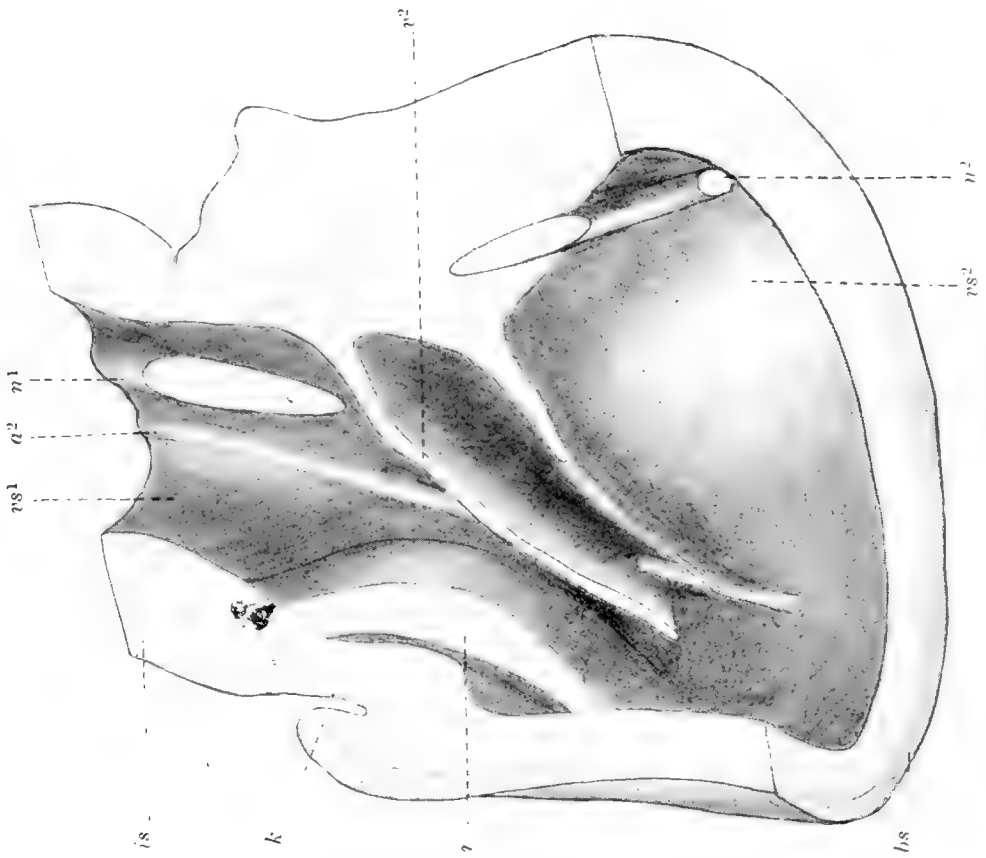


FIG. 2.

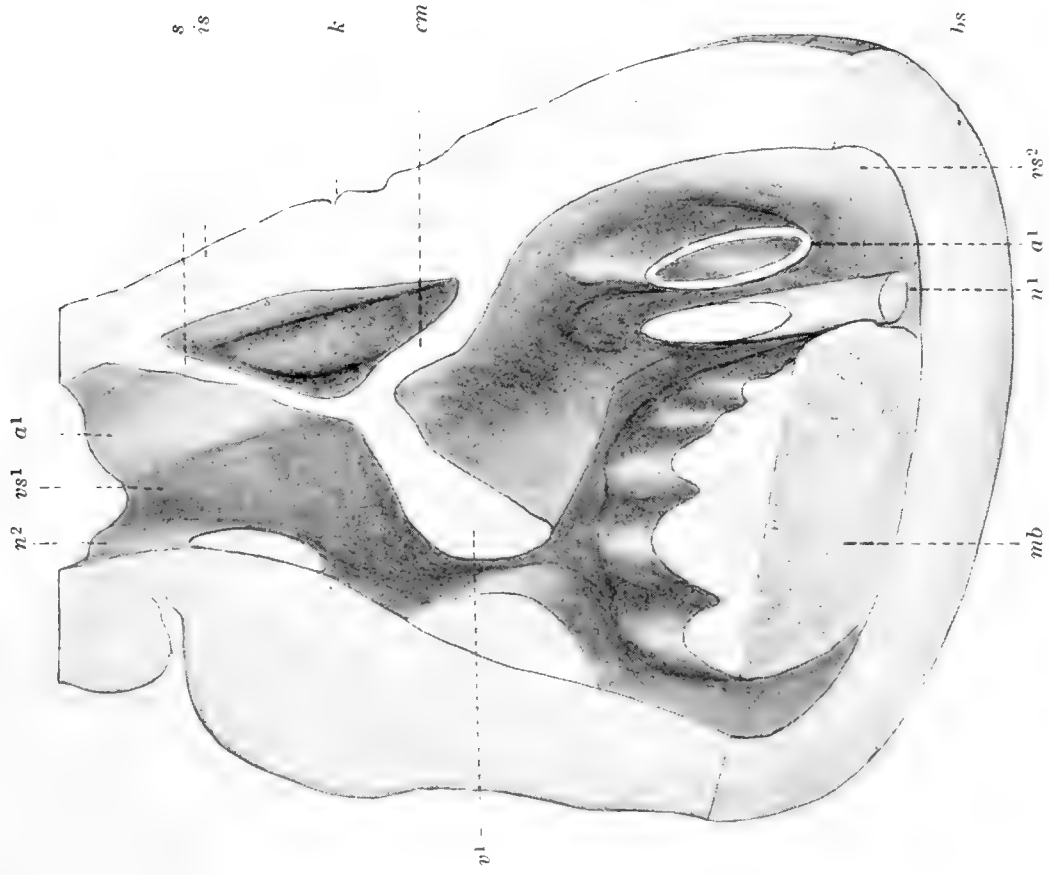


FIG. 1.

PLATE II.

STRUCTURE OF THE CHELA IN THE REGION OF AMPUTATION.

FIG. 3. Section of the breaking joint from the region midway between the parts represented in Figs. 1 and 2, to show the relations of the epidermis, nerve trunk, and connective tissue membranes preceding autotomy. × 368.

FIG. 4. Showing relation of tissues after the autotomy of the limb, and the function of the valve (v^2) in closing the venous blood channel (vc). × 368.

FIG. 5. Section showing the relations of the muscle (m) discovered at the breaking joint (bk). × 204.

FIG. 6. In the fifth stage lobster this muscle has almost entirely degenerated only remnants (md) of the former muscle being present. × 210.

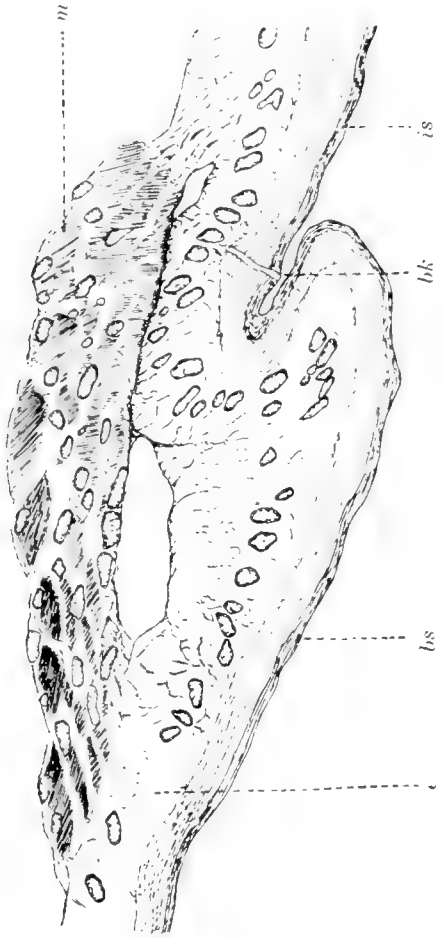


Fig. 5.

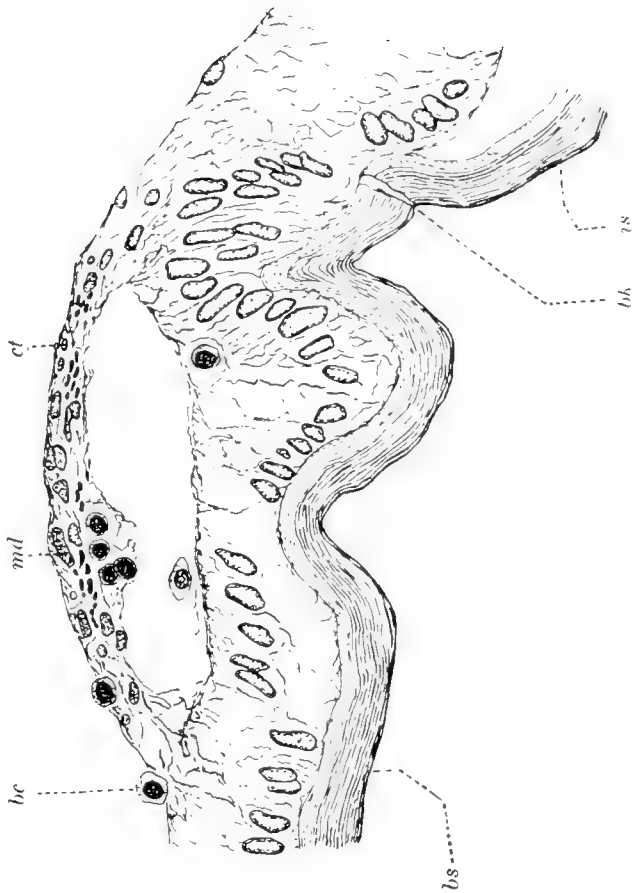


Fig. 6.

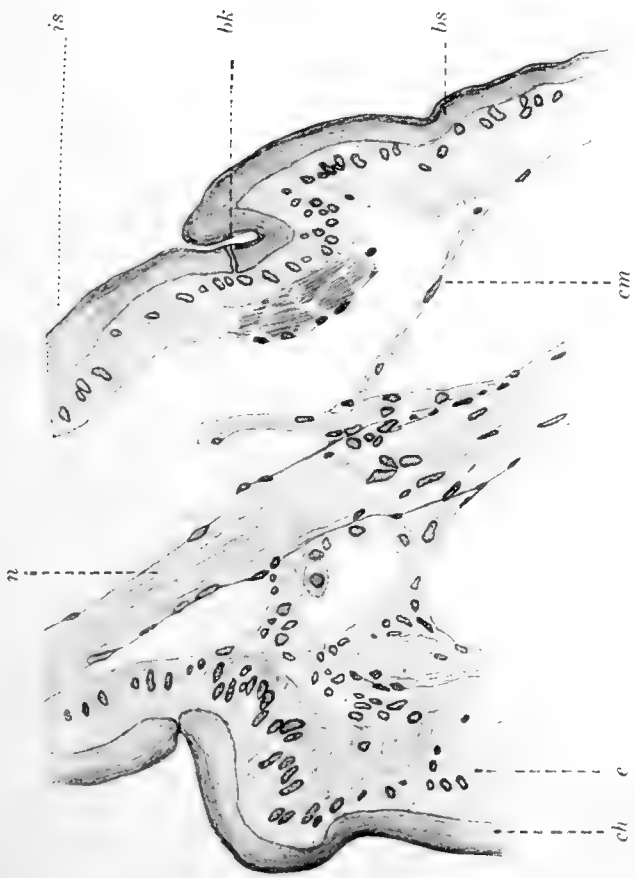


Fig. 3.

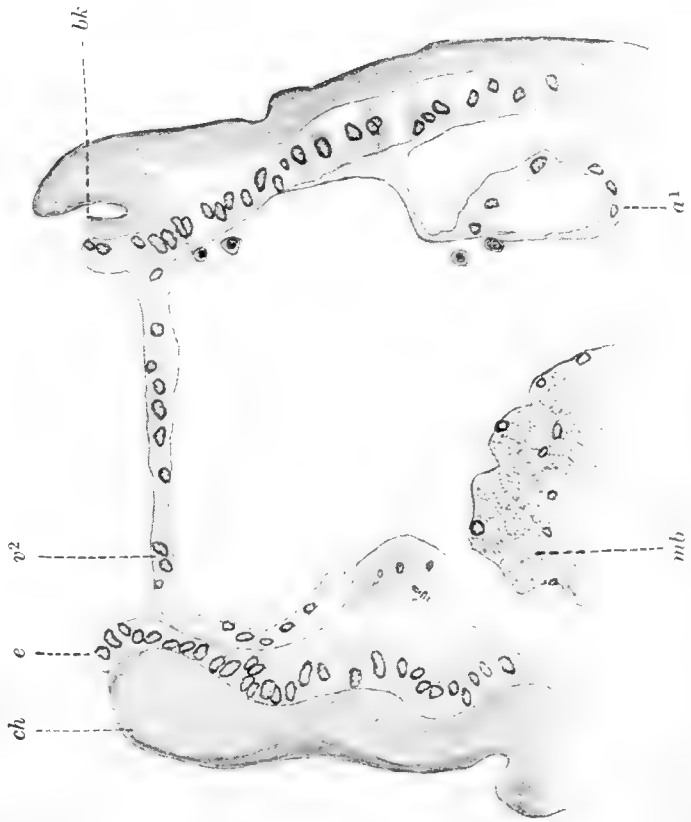


Fig. 4.

PLATE III.

SUCCESSIVE STAGES IN THE REGENERATION OF THE LIMB.

FIG. 7. Regeneration, 1 day. Epidermal cells (e^1) beginning to migrate across the wound beneath the blood clot (*bcl*). × 240.

FIG. 8. Regeneration, 1 day, 14 hours. Migrating epidermal cells have formed a complete plate or disc over the wound. Valve (*v*) of the venous blood channel now assuming its normal position. × 240.

FIG. 9. Regeneration, 2 days, 2 hours. The beginning of cell multiplication (*mt*) in the first formed epidermal plate. × 240.

FIG. 10. Regeneration, 2 days, 22 hours. Epidermal cells (*cc'*) at the apex of the bud are migrating inward to form the flexor muscle of the claw. × 240.

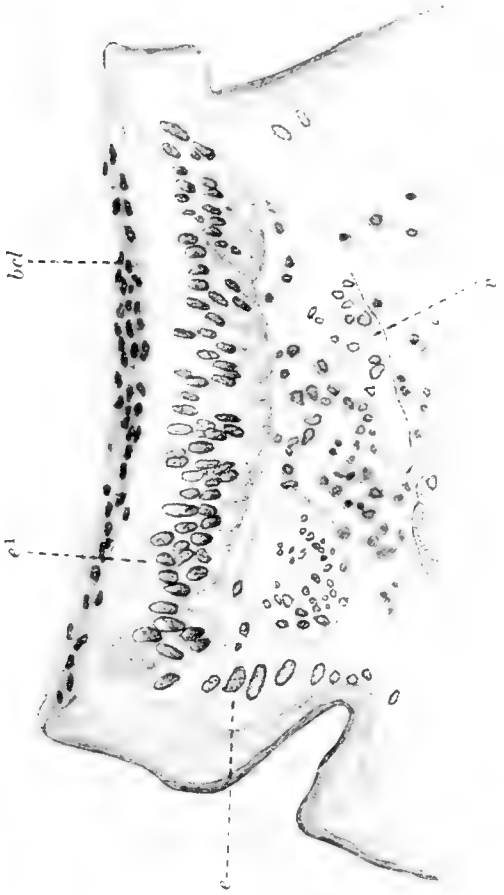


FIG. 8.

FIG. 7.

FIG. 10.

FIG. 9.

PLATE IV.

Nuclear Changes.

Changes in epidermal nuclei during regeneration. The groups of nuclei in Figs. 11-13 taken from the same region of the limb, *i. e.*, on the same side of the limb just below the breaking joint; in each case (*c*) and (*d*) are nearer the center of the wound than (*a*) and (*b*).

Fig. 11. Normal nuclei before the amputation of the limb. × 2000.

Fig. 12. Regeneration, 14 hours. × 2000.

Fig. 13. Regeneration, 24 hours. × 2000.

Fig. 14. Regeneration, 2 days, 2 hours. Nuclei undergoing mitosis; *a* (apparently), prophase; *b*, anaphase. × 2000.

Fig. 15. Regeneration, 5 days, 10 hours. Nuclei in the epidermal wall of the regenerating limb bud, in the region of the invagination (*in*) for the flexor muscle in the meropodite. × 1250.

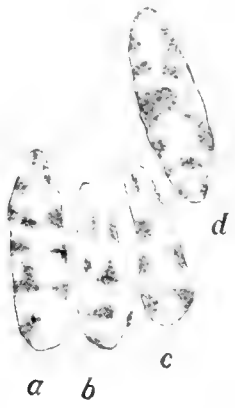


FIG. 11.

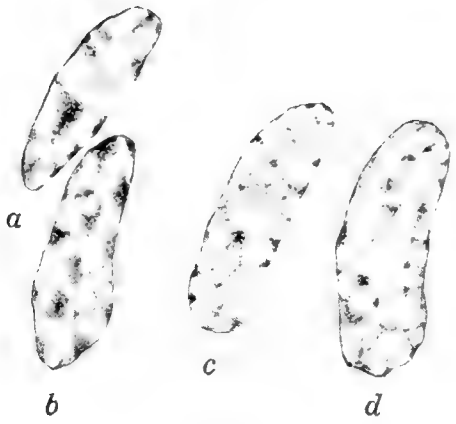


FIG. 12.

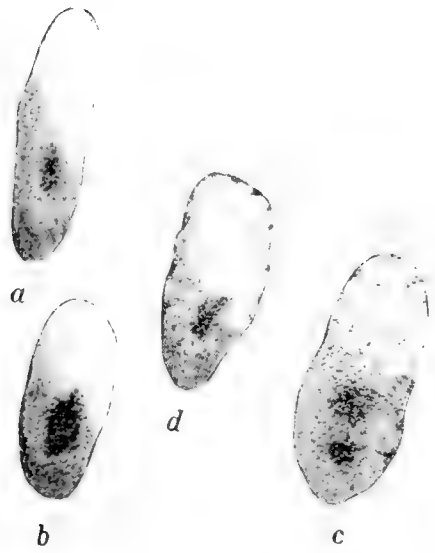


FIG. 13.

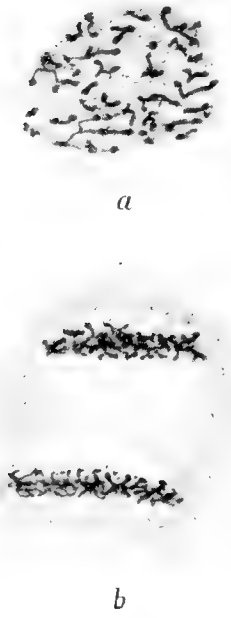


FIG. 14.

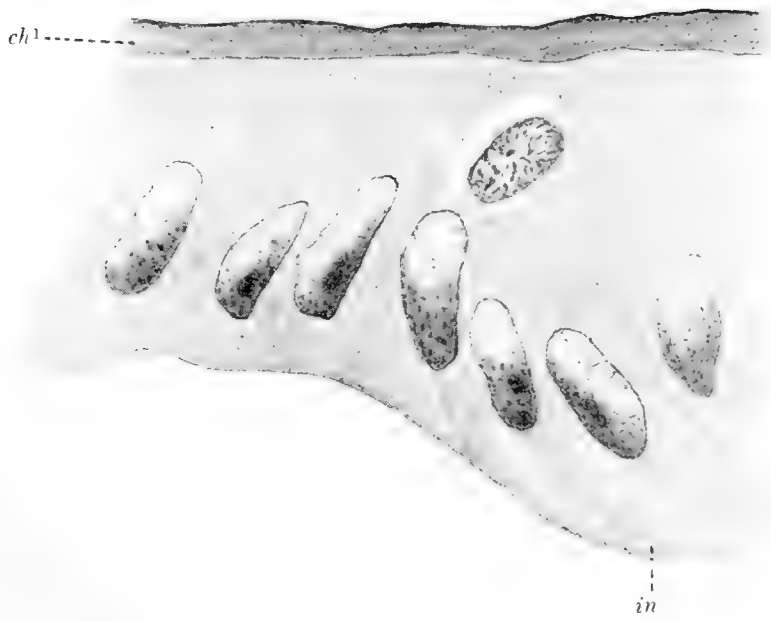


FIG. 15.

PLATE V.

Regeneration of Striated Muscle.

Fig. 16. Regeneration, 6 days, 6 hours. Myofibrillæ (*mf*) just beginning to differentiate for the flexor of the dactylopodite. × 334.

Fig. 17. Regeneration, 7 days, 6 hours. Showing the great elongation of epidermal nuclei during their invagination for the formation of the muscles,—in this case the flexor is the meropodite. × 1250. (Cf. with Fig. 15.)

Fig. 18. Regeneration, 12 days, 10 hours. Shows striation of muscle fibrils (*mf*) and their attachment to the chitin of exoskeleton. × 334.

Fig. 19. Showing skeletal attachment of regenerating muscle fibrils. (From the regenerating chela of a two-year-old lobster.) The myofibrillæ appear to be attached directly to the chitin (*ch'*). × 292.

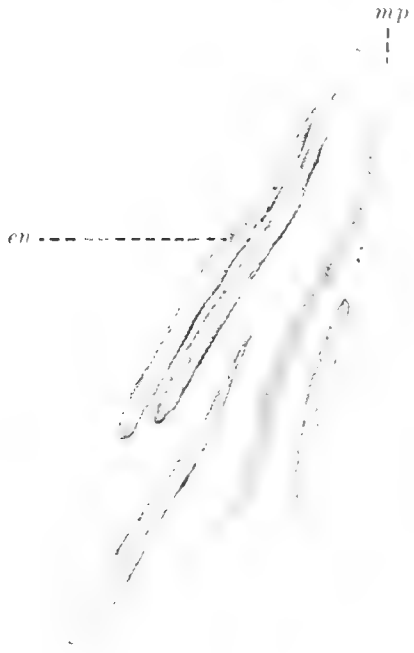


FIG. 17.

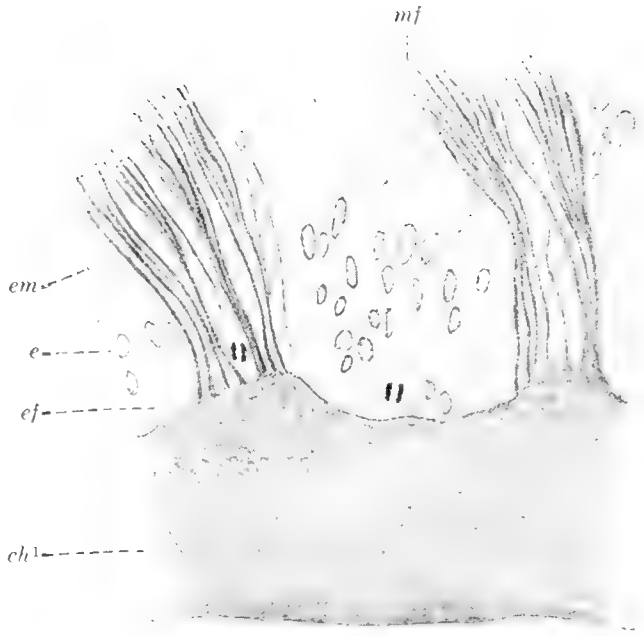


FIG. 19.

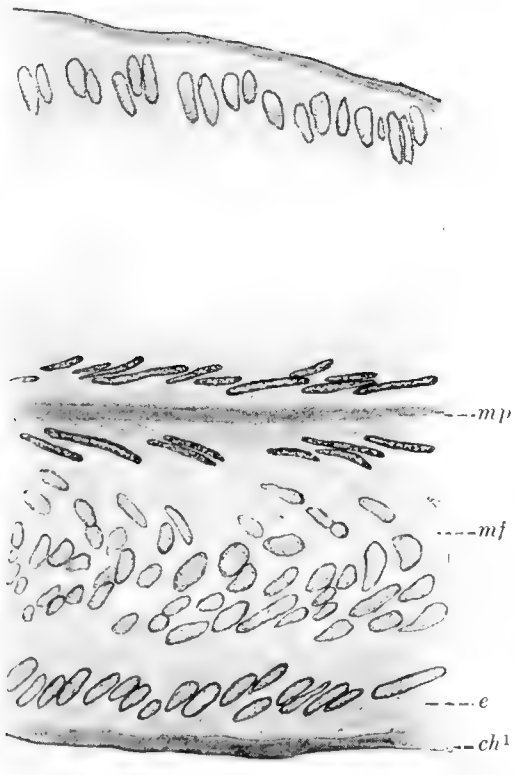


FIG. 16.

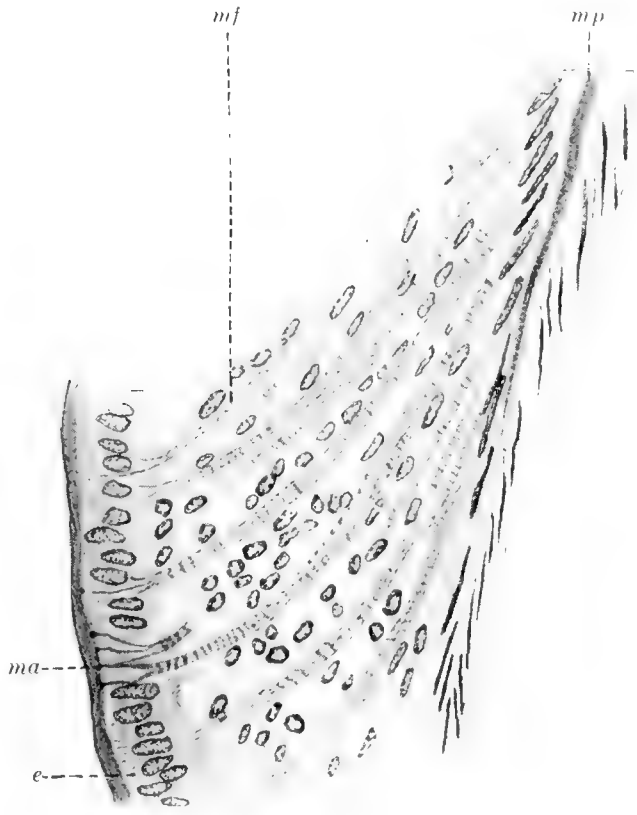


FIG. 18.

PLATE VI.

Regeneration of Striated Muscle.

Fig. 20. Regeneration, 10 days, 6 hours. Showing the muscle fibrils forming brush-like end pieces in their attachment to the chitin. Epidermal membrane (*em*) just beginning to differentiate. × 1250.

Fig. 21. Completely regenerated epidermis in the chela of a sixth stage lobster just after moulting, showing a nucleus (*ng*) of the "grenzlamelle," and the relation of the "grenzlamelle" to the epidermis and muscle fibers. × 750.

Fig. 22. Regeneration, 6 days, 2 hours. Showing the close relation of the myofibrillae (*mf*) to the cytoplasmic reticulum (*rt*) during early differentiation. × 1250.

Fig. 23. Regeneration, 7 days, 6 hours. Myofibrillae (*mf*) surrounded by a sheath of modified cytoplasm (transverse section). × 1250.

Fig. 24. Regeneration, 8 days, 10 hours. Transverse section of myofibrillae (*mf*), showing the first longitudinal splitting of the fibrils. × 1250.

Fig. 25. Regeneration, 11 days, 10 hours. Transverse section of a fully formed muscle fiber showing membrane or sarcolemma (*sa*), nuclei, and Cohnheim's areas. × 1250.

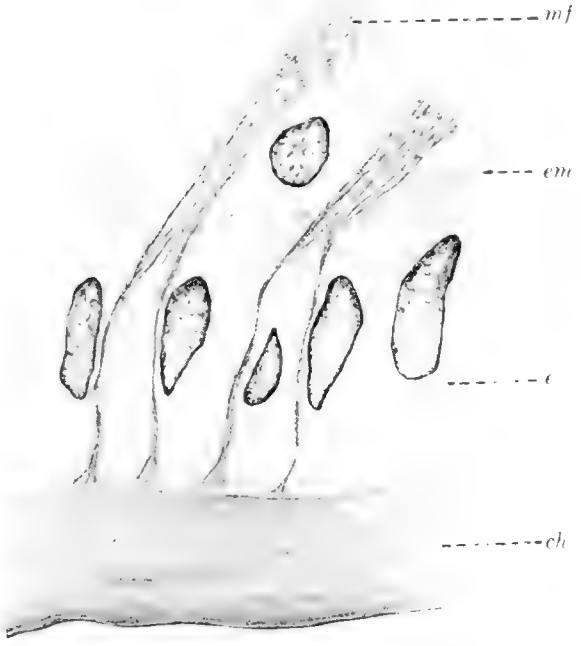


FIG. 20.

FIG. 21.

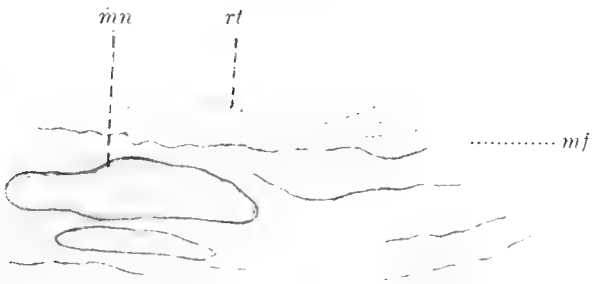


FIG. 22.

FIG. 23.

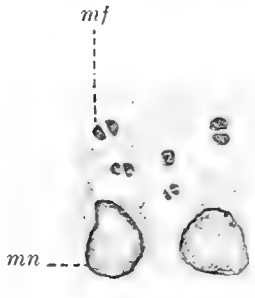


FIG. 24.

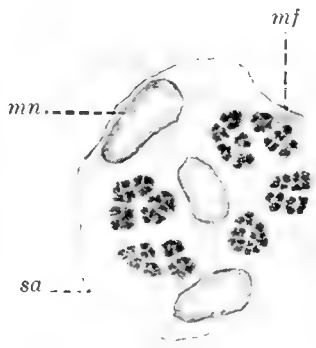


FIG. 25.

PLATE VII.

Regeneration of Connective Tissue and Nerves.

Fig. 26. Regeneration, 10 days, 2 hours. Shows inward proliferation of epidermal cells to form a sheet of connective tissue (*ic*) across the segment (meropodite). × 417.

Fig. 27. Regeneration, 11 days, 10 hours. A later stage in the differentiation of the connective tissue (in the meropodite) showing vacuolation and infiltration of blood plasm (*b*). × 334.

Fig. 28. Regeneration, 5 days, 10 hours. Proliferation of epidermal cells (*c*) in the formation of a nerve fiber (*n*). × 582.

Fig. 29. A stage in the differentiation of the nerve fiber showing a nucleated sheath and axial core of delicate neurofibrillæ (*nf*) in the axis cylinder. At the periphery of this core of fibrillæ may be observed two heavier fibrillæ. × 750.

Fig. 30. A stage of the regenerating nerve fiber in which the heavier neurofibrillæ predominate. × 750. (Figures 29 and 30 were made from sections of the regenerating chela of a two-year-old lobster in which the neurofibrillæ were more sharply defined than in preparations from younger specimens.)

FIG. 26.

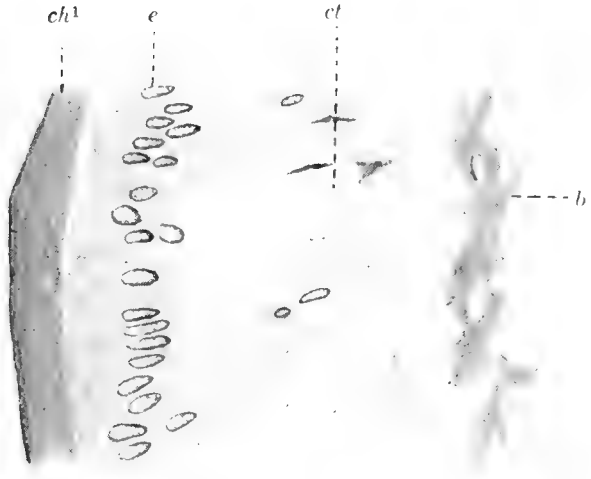


FIG. 27.

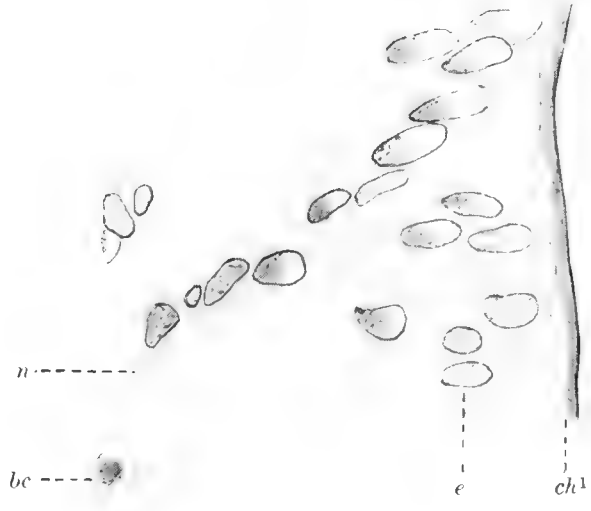


FIG. 28.

FIG. 30.

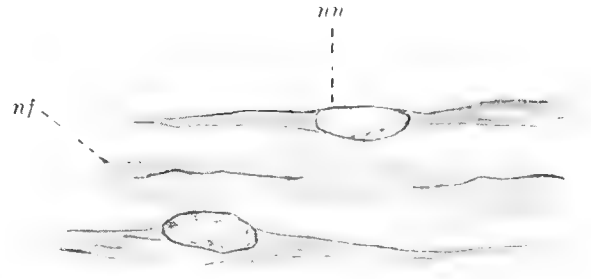


FIG. 29.

PLATE VIII.

The Direction of Differentiation.

The direction of differentiation of the eight chitinogenous muscle plates or tendons of the limb. Figs. 31-36 are reconstructions from serial sections.

Fig. 31. Regeneration, 2 days, 22 hours. × 60.

Fig. 32. Regeneration, 4 days, 6 hours. × 60.

Fig. 33. Regeneration, 4 days, 22 hours. × 60.

Fig. 34. Regeneration, 5 days, 6 hours. × 60.

Fig. 35. Regeneration, 5 days, 9 hours. × 60.

Fig. 36. Regeneration, 10 days, 2 hours. × 54.

Fig. 37. Diagrammatic drawing of a fully developed chela showing the relative size and position of the eight limb muscles distal of the breaking joint (*bk*). × 4-9.

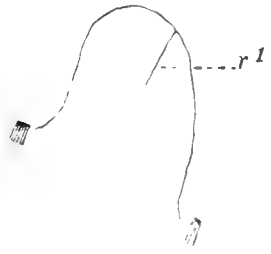


FIG. 31.

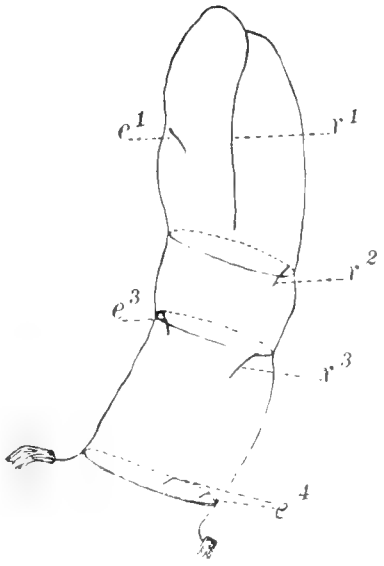


FIG. 34.

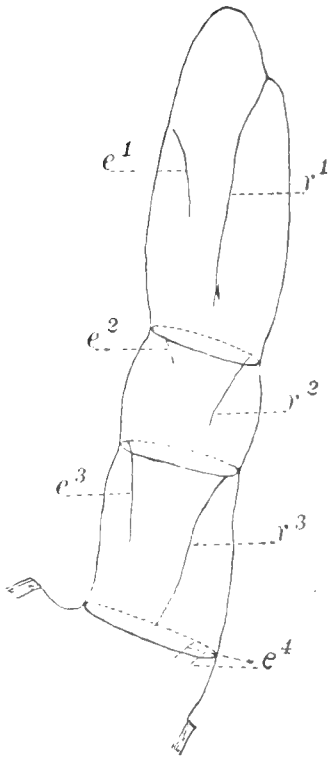


FIG. 35.

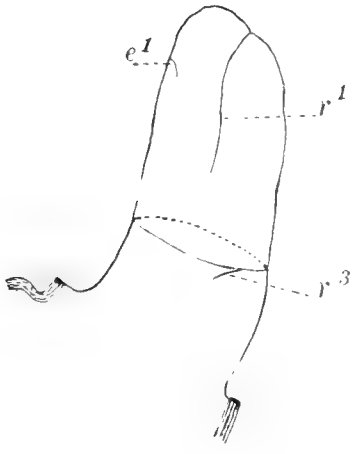


FIG. 32.

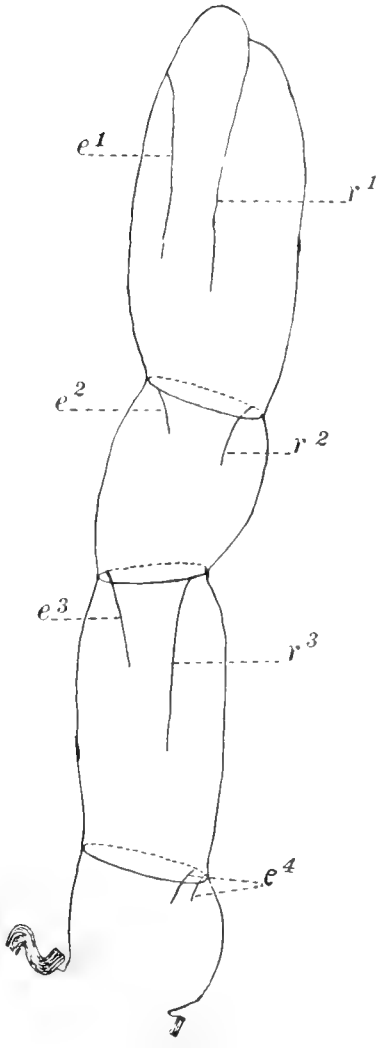


FIG. 36.

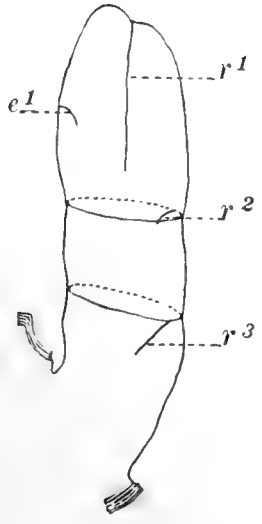


FIG. 33.

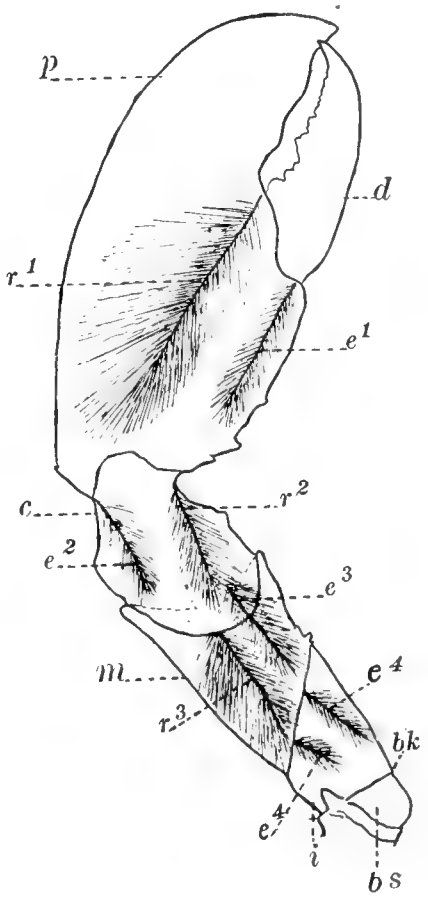


FIG 37.

SOME ANOMALIES IN THE GENITAL ORGANS OF BUFO LENTIGINOSUS AND THEIR PROBABLE SIGNIFICANCE.

BY

HELEN DEAN KING,

Assistant in Anatomy, Wistar Institute.

WITH 26 FIGURES.

During the course of a series of investigations on the germ-cells of the common American toad, Bufo lentiginosus, I have had occasion to examine a large number of these amphibians at various stages of their development, and I have found many individuals in which the genital organs showed marked deviations from the normal type. The most striking of these anomalies are described in the present paper. Many cases of this kind have a direct bearing on the question of the existence of hermaphroditism in the primitive vertebrates, and but few of them have as yet been recorded for any amphibian other than Rana.

I. ANOMALIES IN THE GENITAL ORGANS OF YOUNG TOADS.

Anomalies occur much more commonly in the sex-glands of young toads than in those of adults, and at least two per cent of the young individuals that I have examined showed more or less marked irregularities of this kind. As most of the toads in which abnormalities were found were reared in the laboratory, one might infer that the anomalies were the result of pathological changes produced in the genital organs by abnormal environmental conditions. That such is not the case, however, is shown by the fact that eleven individuals in which the sex-glands were anomalous in some respect were found in a lot of 500 young toads that had completed their metamorphosis under natural conditions.

Although in Bufo sex is probably determined at or before the time that the egg is fertilized, the gonads are often in an apparently

indifferent state even at the time of metamorphosis, and in many instances the genital glands must be examined histologically before the sex of an individual can be ascertained. The toad grows very rapidly after completing its metamorphosis, and then, except in very rare cases, the sexes are readily distinguished by an examination of the sex-glands under a dissecting lens. At this period of development the testes are long, cylindrical bodies with a smooth contour (Figs. 3 and 6, Left); the ovaries, although they are of about the same length as the testes, are relatively broader and they have an irregular, jagged outline (Figs. 5 and 7, Left). At the anterior end of each genital gland is a large rounded body, Bidder's organ (Fig. 1, B. O.), to which the corpora adiposa are attached (Fig. 1, C. A.). In young toads Bidder's organ is of the same size and structure in both sexes: it persists throughout the lifetime of the male, but it disappears in the female towards the end of the second year. A study of the structure and development of Bidder's organ (King, '08 a) has shown that this body is undoubtedly a rudimentary ovary in which the cells have marked characteristics which readily distinguish them from the eggs developing in the ovaries.

As a rule there is but one Bidder's organ at the anterior end of a sex-gland, and it usually measures about 0.4 mm. in diameter. In many young toads Bidder's organ shows considerable deviation from the normal size and shape. In some instances this structure is very large, measuring 0.6 mm. in diameter (Figs. 1, 2, 7); in rarer cases it is less than one-half of its usual volume (Fig. 13). Examined histologically such Bidder's organs are found to differ from those of normal size only in the number of ova that they contain.

Not infrequently a Bidder's organ is found that is greatly elongated (Figs. 7, 9, 12). In such cases this body is usually indented in the middle region (Figs. 7, 9), although sometimes it has a perfectly smooth contour (Fig. 12). Anomalies of this kind indicate clearly the steps by which the one Bidder's organ becomes divided into two; and the presence of two of these structures at the anterior end of an ovary or of a testis is probably the most common abnormality occurring in the genital organs of young toads. Usually, in cases like this, one Bidder's organ lies directly behind the other (Figs. 1, 10, 11,

14); more rarely these bodies are separated as shown in Fig. 3. I have found one instance only in which there were three Bidder's organs at the anterior end of a sex-gland (Fig. 16). Abnormalities of this type can doubtless be ascribed to the mechanical effect of pressure exerted by some structure on Bidder's organ at a very early period in its development.

In many individuals one or more rounded bodies, similar in appearance to the normal Bidder's organ although usually much smaller, appear along the course of the sex-gland (Figs. 1, 2, 5, 8, etc.). Structures of this kind may occur on any part of the gland, and they are found in both sexes, although they are, perhaps, more common in males than in females. The probable origin of these bodies will be considered later.

Fig. 12 shows in outline the most interesting of the anomalies found in the genital organs of young toads. On the left side Bidder's organ appears much enlarged and greatly elongated; on the right there are four rounded Bidder's organs lying one behind the other. Sections of these bodies show that each has the structure typical of a normal Bidder's organ. As in this individual the ovaries were not more than one-half of their normal length, it is evident that some of the germ-cells in the anterior part of each ovary became incorporated with Bidder's organ and assumed the characteristics of rudimentary ova. A somewhat similar case in which the anterior part of a testis has been changed into a Bidder's organ is shown in Fig. 16. Abnormalities of this kind can hardly be due to any mechanical cause, although possibly a lessening of the normal blood supply to the anterior part of a sex-gland might induce such changes which undoubtedly must be considered as degenerative in their character.

In all of the cases so far described, and in many others of the same general character, sections were made of the genital glands and the anomalies appearing in them carefully studied. All enlargements of the sex-glands, no matter what their position or their size, were found to contain large cells having all of the characteristics of the rudimentary ova of which the normal Bidder's organ is composed. If there are two Bidder's organs at the anterior end of a sex-gland,

each has the structure typical of the normal Bidder's organ. Most of the large bodies found along the course of the sex-glands (Figs. 6, 13, 16) also appear similar in structure to Bidder's organ, and they are directly connected above and below with germ-cell tissue that appears normal in every respect.

The structure of many of the smaller bodies found on the sex-glands does not conform strictly to that of Bidder's organ. Fig. 17 shows a longitudinal section of the enlargement on the lower part of the left ovary, which is outlined in Fig. 10. The ovarian wall has a perfectly normal structure here as in other parts of the sex-gland, being composed of cysts of secondary oögonia and of oöcytes in the synizesis stage of development (Fig. 17, S.). In the cavity of the ovary is a mass of large cells inclosed by follicle cells and a thin membrane. These large cells have all of the characteristics of the large rudimentary ova normally found in Bidder's organ; they do not bear the slightest resemblance to the oögonia and oöcytes of which the ovarian wall is composed. A somewhat different arrangement of tissues is shown in Fig. 18 which is a drawing of a transverse section through the smaller of the two bodies on the right testis outlined in Fig. 2. Here the spermatogonial tissue does not inclose the group of cells which appear like rudimentary ova, but is collected together at one side of it and the cells are surrounded in great part only by a thin covering of mesentery. A similar condition of the tissues was found in the enlargements of the testes shown in outline in Figs. 8 and 15.

As a rule only the enlarged portions of the sex-glands contain any of the large cells which appear like rudimentary ova, in all other parts the glands have a normal structure. In but one instance have I found cells of this character among germ-cells when their presence was not shown by an examination of the sex-gland under a dissecting lens. In this case the cavity of one of the ovaries in a young female contained three of these large cells which were separated a considerable distance and thus gave no external evidence of their presence.

During the course of my investigations on the toad (King, '07, '08, '08a) I have made sections of the gonads of a large

number of tadpoles in various stages of development from the time of hatching up to metamorphosis. In no case have I found any abnormalities in the gonads proper, although in several instances Bidder's organ on one or both sides had been divided as shown in Figs. 11 and 14. The large cells resembling rudimentary ova which are found singly or in groups in the genital organs of so many young toads that have recently completed their metamorphosis must, therefore, develop very quickly, presumably just before or during the period of metamorphosis. It is highly improbable that these cells originate in Bidder's organ and subsequently migrate into the sex-gland, as there is never any opening between these structures except in the female toward the end of the second year when the entire Bidder's organ is degenerating. Such cells, moreover, never show the slightest evidence of amœboid movement, and in many instances the membrane surrounding them is continuous with that inclosing the primordial germ-cells themselves. Judging from my previous investigations I am strongly inclined to the opinion that these cells are primordial germ-cells in which, for some unknown reason, the course of development has been changed so that the cells increase in size with unusual rapidity and assume the characteristics of rudimentary ova. Cells of this kind must, therefore, be considered as degenerating cells. Their presence in the sex-gland is apparently not harmful to the individual, since none of the young toads in which they are found seem to differ in any other way from the normal type. In Bufo, with rare exceptions, all cells of this character must become absorbed early in the life history of the individual. I have never found any of them in the sex-glands of toads that were more than three or four months old.

According to Pflüger, '82, there are three kinds of individuals to be found among young frogs killed soon after completing their metamorphosis: males, females, and hermaphrodites. In the course of a few months the hermaphroditic forms become either definite males or females, and in few cases only does the hermaphroditic condition persist until the individual becomes an adult. During the course of a series of investigations on the determination of sex in frogs, Hertwig, '06, '07, has found a number of individuals in which sex

could not be ascertained even after metamorphosis was completed. These individuals, Hertwig believes, correspond to the "hermaphroditic" forms described by Pflüger. Schmitt-Marcel, '08, has recently studied the structure of the sex-glands in young Rana temporaria that appeared to be hermaphrodites. He states that in all cases the sex-glands of such individuals, which he calls "intermediate forms," appear much more like ovaries than like testes, yet they probably all ultimately develop into testes. In accounting for the origin of such forms Schmitt-Marcel assumes that they are derived from young females, and he concludes: "Die Veränderung also, die von einer normalen weiblichen Drüse zu dieser Bildung führt, besteht im wesentlichen wohl darin dass bei einem Wachstum des Organes nicht in der ganzen Keimdrüse ein Heranwachsen von Urkeimzellen zu jungen Eizellen stattfindet, sondern dass ganze Strecken auf dem Stadium der Urkeimzellen stehen bleiben und sich als solche weiter vermehren, während gleichzeitig eine ausserordentliche in der normalen weiblichen Keimdrüse fehlende Vermehrung der Stroma Platz greift." The young ova grow to a considerable size and then degenerate; meanwhile indifferent germ-cell tissue spreads throughout the whole sex-gland and later develops into spermatogonia so that the individual eventually becomes a male. Schmitt-Marcel states that he finds an increasing number of such forms among young frogs killed from the second to the tenth month after metamorphosis, and that the number of these forms gradually decreases after this time. Among adult frogs such "intermediate forms" are not known, males and females being found in about equal proportions.

There is a great similarity between the sex-glands of the "intermediate forms" found among young Rana temporaria and those of young toads having the anomalies described above. In both cases large cells, having the general appearance of young ova, are found among primordial germ-cells which are still in an apparently indifferent state. These cells develop to a certain size and then undergo processes of degeneration and absorption, leaving the gland male or female as the case may be. The chief differences between such anomalies in Rana and those in Bufo consist in the fact that the large cells which appear in the sex-glands of young toads are, as a

rule, segregated into one or several masses which can be seen under a low magnification before the gland is sectioned, and they have very distinctive characteristics that distinguish them from normal ova; in Rana, according to Schmitt-Marcel, the large cells are scattered throughout the sex-gland, and they appear in every respect like normal ova.

Schmitt-Marcel offers no suggestion as to how or why, in an individual in which the female sex is already determined, the primordial germ-cells can change the course of their development and subsequently alter the sex of the individual. If this phenomenon is of common occurrence in young Rana temporaria, then it would seem as if some clue might be obtained as to the causes which determine sex in this species if an extensive series of experiments was to be made with young individuals that had recently completed their metamorphosis.

Except in rare cases, the sex of young toads in which anomalies appear in the genital organs can readily be ascertained, since, although the primordial germ-cells may appear alike in both sexes at this time, the ovary has a central cavity which is lacking in the testes. There are, therefore, no young toads that can properly be called "intermediate forms," as each individual is at this time definitely male or female, no matter how many cells appearing like rudimentary ova may be present in the sex-glands.

The development of the genital organs must be considerably slower in Rana temporaria than in Bufo lentiginosus, since in the former species it is impossible to ascertain the sex of many individuals until they are nearly a year old. As according to Schmitt-Marcel the germ-cell tissue which spreads throughout the sex-glands in these intermediate forms is still in an indifferent state I can see no valid reason for the assumption that such forms were originally females. In Bufo, as far as I can judge from the number of cases (about 75) that have come under my observation, large cells with the characteristics of rudimentary ova appear somewhat more commonly in the genital glands of young males than in those of young females. Assuming that a similar condition exists in young Rana temporaria, one readily accounts for the fact that the great majority

of "intermediate forms" ultimately become males, and one also avoids the necessity of presuming that sex can be altered after an individual has completed its metamorphosis.

In young toads, and possibly also in young frogs, primordial germ-cells that fail to undergo normal processes of development assume the characteristics of rudimentary ova, regardless of the sex of the individual in which they occur. An explanation of this phenomenon seems to me possible if one accepts for the amphibians Haeckel's, '74, view "dass das älteste und ursprünglichste Geschlechtsverhältniss die Zwitterbildung war und dass aus dieser erst secundär (durch Arbeitstheilung) die Geschlechtstrennung hervorging." In the male amphibian at the present time many of the primordial germ-cells still have the power, under certain conditions, of developing into ova which, since they cannot leave the sex-gland or come to maturity, are destined to degeneration and absorption. Except in very rare cases the primordial germ-cells in the female are no longer able to develop into spermatogonia. When, therefore, these cells fail to develop along normal lines they become rudimentary ova which are similar in structure to the rudimentary ova derived from the primordial germ-cells in the male and they have a similar fate.

In adult amphibians, as a rule, germ-cells which fail to undergo normal processes at any stage of their development disintegrate at once and become absorbed. Some few cases have been found, however, in which germ-cells in adult males have changed the course of their development and become rudimenary ova. Cole, '95, Friedmann, '98, Latter, '90, and Punnett, '00, have noted the presence of rudimentary ova in the testes of various species of frogs, and I also have a preparation of the testis of an adult Rana pipiens which shows this anomaly. Rudimentary ova have also been found in the testes of adult Bufo vulgaris by Spengel, '76, Hoffmann, '86, Knappe, '86, and Friedmann, '98, but as yet no cells of this kind have been found in the testes of the American species, Bufo lentiginosus.

Fig. 4 shows a type of abnormality which is found occasionally in the sex-glands of young toads. The median genital ridge, which is usually divided into two ridges when a tadpole is from twelve to

fourteen days old, has separated only in the anterior region and it still remains undivided posteriorly even after the toad has completed its metamorphosis. Sections of the sex-glands show no other apparent abnormality.

II. Anomalies in the Genital Organs of Adult Toads.

Among the large number of adult toads which I have examined during the past ten years there was but one individual in which the sex-glands appeared in any way abnormal. This toad, which proved to be a rudimentary hermaphrodite, was brought into the vivarium of the University of Pennsylvania on the morning of March 30, 1907, having been captured in a pond near West Philadelphia. The abnormal character of the sex-glands was noticed as soon as the body cavity was opened, and these organs were removed at once and fixed in Flemming's fluid; the body of the toad being placed in a 4 per cent formalin solution for future examination.

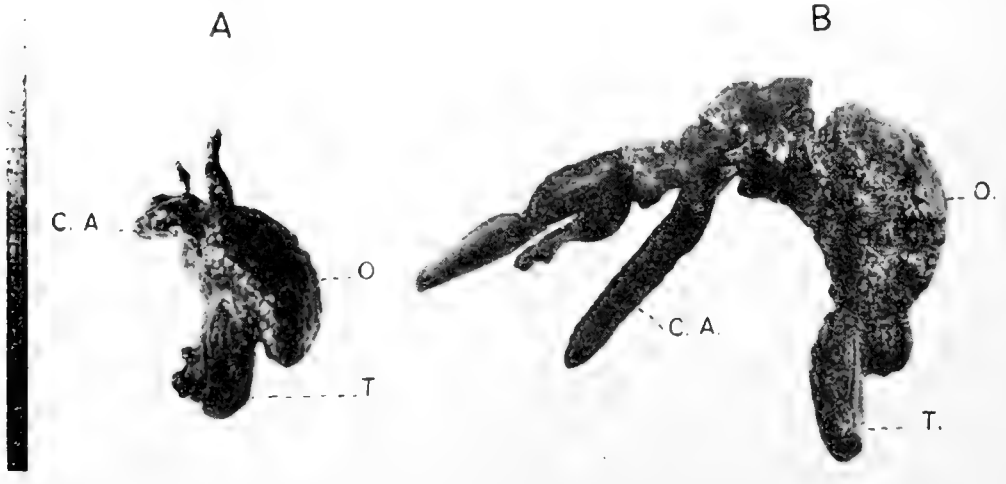
Measurements that were made showed that this toad was somewhat larger than the average male as it had a body length of 8.5 cm. The thumbs were deeply pigmented, and they bore pads similar to those found on the thumbs of all male toads during the breeding season, although these structures were somewhat smaller than normal.

Photographs of the genital organs of this individual are shown in the text-figure. On the left side (B) there is a well developed testis (T), measuring 8 mm. in length and 3 mm. in width. Above the testis is a large, pigmented, irregularly shaped body (O), unquestionably a rudimentary ovary, which is 15 mm. long and 7 mm. across at its widest part. The genital organs on the right side (A) are noticeably smaller than those on the left, although they have a similar structure. The testis (T) is but 7 mm. long; the rudimentary ovary above it (O), which has a much more regular outline than that on the left side, measures 10 mm. in length and 7 mm. in width. There is no Bidder's organ on either side, the fat bodies (C. A.) being attached directly to the anterior surface of the rudimentary ovaries.

Transverse sections through the testes show that these bodies are composed of cysts filled either with ripe spermatozoa or with sper-

matocytes and spermatids in various stages of development. A careful study of many cysts failed to show a single instance in which the development of the germ-cells appeared to be abnormal. The cysts in the centre of the testes are not crowded quite as closely together as are the cysts in the testes of the normal male, and the stroma separating them is unusually thick; in all other respects the testes appear perfectly normal. Each testis was connected with the kidney by vasa efferentia, the genital ducts appearing normal. In this individual the Müllerian ducts were still present; but, although they were quite large and much convoluted, they showed no dilatations either in the anterior or in the posterior region.

Interest in this individual centers in the structure of the rudimentary ovaries lying above and attached to the testis on either side.



Photographs of the right (A) and of the left (B) ovo-testis found in an adult Bufo lentiginosus. O, ovary; T, testis; C. A, corpora adiposa.

The position of these bodies and the fact that no Bidder's organs are to be found indicate that they have been produced, in part at least, from the cells which ordinarily form Bidder's organ. Since in this individual the testes are somewhat shorter than those usually found in adult males, it seems probable that primordial germ-cells in the anterior part of the germinal ridge, which normally would have developed into spermatozoa, joined with the cells of Bidder's organ to form these rudimentary ovaries. The force, whatever its nature, which modified the development of the cells and brought about the formation of these rudimentary ovaries must have acted

at a very early period in the life history of the individual, as in a normal toad the cells which develop into Bidder's organ begin to show characteristics which distinguish them from the other germ-cells when a tadpole is about two weeks old.

Sections show that each ovary contains a well defined central cavity which is lined by epithelial cells, and that the structure of the ovarian wall is the same in each case. In both ovaries the great majority of the ova are practically of the same size and in about the same stage of development. It is not possible, therefore, to trace the various stages in the growth of the cells or to discover any apparent reason for their unusual mode of development. With but a few exceptions the cells in both ovaries have developed uniformly along the same lines, and those in the upper part of each ovary bear no more resemblance to the cells which are typical of Bidder's organ than do the ones lying more posteriorly which were derived, presumably, from primordial germ-cells belonging to the sex-gland proper.

The smaller ova, which usually lie at the periphery of the ovaries, are rounded in outline, and they have an average diameter of 0.14 mm. As a rule, the cytoplasm of these cells appears uniformly granular, although sometimes it contains yolk nuclei, as shown in Fig. 19, Y. N. The nuclei are round, or slightly oval, and they measure about 0.08 mm. in diameter; with but few exceptions, all of them are in the early post-synizesis stage of development (Fig. 22). I can detect nothing in the structure of the great majority of these small cells that would in any way serve to distinguish them from normal young oöcytes of the same size.

In some few cases the smaller cells of these rudimentary ovaries exhibit all of the characteristics of the young ova normally found in Bidder's organ. A section of a cell of this type is shown in Fig. 19. The nucleus contains a number of nucleoli of various sizes and chromatin threads which are composed of a series of deeply staining, rounded granules. Two of the larger nucleoli show degenerative changes that are similar to those taking place in the large nucleoli of the cells of Bidder's organ which are beginning to disintegrate. The body of the cell contains a number of finely granular masses,

sharply distinct from the cytoplasm, which stain very intensely with iron hæmatoxylin (Fig. 19, Y.N.). These are the so-called "yolk-nuclei" which are always present in the normal ova at certain stages in their development, and which sometimes appear in the young cells of Bidder's organ. The arrangement of the yolk-nuclei in the cell shown in Fig. 19 is very similar to that of the yolk-nuclei in the cells of Bidder's organ. I have not been able to find any cells in these rudimentary ovaries that might be considered as intermediate in structure between normal and rudimentary ova.

The larger cells in the rudimentary ovaries border the central cavity, and, owing to pressure, they are usually greatly distorted in shape. The average diameter of these cells is 0.6 to 0.7 mm.; while the diameter of their nuclei ranges from 0.17 mm. to 0.25 mm. As a rule the nuclei of these cells resemble to a remarkable degree those of young ovarian ova of the same size: they are rounded in outline and contain a large number of scattered nucleoli; the karyoplasm appears uniformly finely granular; and the chromatin threads have the feathery structure characteristic of the chromosomes in the young ovarian oöcytes (Fig. 25).

The structure of the cell body in many of these larger cells differs considerably from that normally found in the ovarian oöcytes of the same size. As a rule the cytoplasm is much vacuolated, as if degenerative processes had already begun in it. In some cases the vacuoles extend radially from the periphery of the cell to the nucleus, giving the cytoplasm a striated appearance when examined under a low magnification; in other cases the vacuolated area extends only around the periphery of the cell and the cytoplasm surrounding the nucleus appears uniformly reticular (Fig. 23). There is a possibility that the vacuolization of the cytoplasm in these cells is due, in part at least, to the way in which the material was preserved. Flemming's fluid, although an excellent fixing agent for the testes at all stages of their development, is but a very indifferent fixative for amphibian ova after they have passed the synizesis stage. The cytoplasm of these large cells would doubtless appear much less vacuolated had some other fluid with greater powers of penetration been used in fixing the ovo-testes.

Many of the ova contain large numbers of yolk spherules which have formed, as in normal ova, at the periphery of the cell. In some few cases I have found two distinct layers of yolk spherules; one lying at the periphery, the other half-way between the surface of the cell and the nucleus (Fig. 24). In cases of this kind it is probable that the inner layer of yolk spherules was derived from yolk-nuclei which appeared in a ring midway between the nucleus and the periphery of the cell and were there transformed directly into yolk spherules. I have found one or two cases somewhat similar to this occurring in the ovarian ova (King, '08).

This hermaphroditic toad was killed at the breeding season when the germ-cells have become mature and the large ova in the normal Bidder's organ have reached the highest stage in development of which they are capable. It seems probable, therefore, that the large cells in the rudimentary ovaries have also attained their maximum development, and that they would have undergone rapid degeneration and absorption to give place to another generation of similar cells, had the individual not been killed. This assumption seems the more plausible since a number of the largest ova in the rudimentary ovaries already show marked degenerative changes.

The processes of degeneration occurring in the nuclei of the large cell found in these rudimentary ovaries are somewhat different from those taking place in the large ova of Bidder's organ, and they differ also from the disintegration processes occurring in mature eggs that for some reason have failed to leave the ovary. The first evidence of degeneration is the migration of the nucleoli to the centre of the nucleus where they lie massed together as shown in Fig. 20. At this time all of the nucleoli are rounded in outline and, with but few exceptions, they stain uniformly black with iron hæmatoxylin. The chromatin threads can sometimes be seen in the nucleus at this stage, although they cannot be found at a later period owing, possibly, to the fact that Flemming's fluid does not fix the chromosomes well in cells of this size. The next step in the degeneration of the nucleus is shown in Fig. 20. Many of the nucleoli appear vacuolated, others stain faintly and are evidently being dissolved. Only that portion of the karyoplasm in which the nucleoli lie is uniformly finely

granular at this time, elsewhere it contains numerous rounded granules, staining very intensely, that are much smaller than the nucleoli and many times larger than the minute granules which normally form the karyoplasm. In a later stage (Fig. 21) the nucleus is filled with numerous short fibres composed of deeply staining, rounded granules which appear similar to those scattered through the nucleus at the stage of Fig. 20. There is no finely granular karyoplasm anywhere in the nucleus at this time, and the fibres, as well as the remaining nucleoli, lie in an apparently fluid space. The immense number of the large granules in the nucleus at this stage of degeneration seems to preclude the possibility that these bodies have been derived from chromatin or from the substance of the relatively few nucleoli that have been dissolved. It seems probable, therefore, that these granules have originated from the granular karyoplasm, since their number increases in proportion as the minute karyoplasmic granules disappear. At the stage of Fig. 21 follicle cells and blood capillaries are beginning to enter the cytoplasm of the cells to complete the processes of disintegration and absorption. The nuclear membrane breaks down at or soon after the stage shown in Fig. 21, and the nuclear contents come in direct contact with the cytoplasm. Unfortunately the rudimentary ovaries contain no later stages in the degeneration of the large ova.

The granular fibres which fill the greater part of the nucleus at the stage of degeneration shown in Fig. 21, bear a very striking resemblance to the "oxychromatin" fibres found in connection with the nucleoli during the early post-synizesis stages in the development of the young oöcytes. It is possible, therefore, that the latter structures are not composed of chromatin but of fused karyoplasmic granules which have great affinity for the chromatin stains. If this interpretation is correct, then the chromatin in the amphibian egg is probably not concerned in any way with the formation of the nucleoli which are doubtless waste products of nuclear metabolism.

Cerruti, '07, has recently given a brief description of two cases of hermaphroditism which he has found in Bufo vulgaris. In one individual the anterior part of each testis had developed into a small rudimentary ovary lying between Bidder's organ and the testis proper:

this case is similar to that found by Spengel, '76, in Bufo cinereus. In the other individual Cerruti found that each Bidder's organ had developed into a rudimentary ovary which contained many large ova in various stages of degeneration and also ova that appeared like normal oöcytes except that they did not have any yolk spherules.

In the second cases noted by Cerruti the condition of the sex-glands is much like that in the hermaphroditic Bufo lentiginosus described above. In both of these individuals it is evident that the rudimentary ovaries were derived from cells which normally would form a Bidder's organ. In young toad tadpoles, as shown in a previous paper (King, '08a), the cells which are destined to form Bidder's organ are directly continuous with the primordial germ-cells that later form the sex-gland; they are similar to the germ-cells in structure, and they develop like the germ-cells up to the synizesis stage. These facts seem to me to prove conclusively that the cells which form Bidder's organ are degenerate germ-cells. It is not surprising, therefore, that occasionally these cells develop into ova which apparently have a normal structure.

Many instances of hermaphroditism have been recorded for various species of frogs. Cases similar to those found in Bufo, where a rudimentary ovary has developed at the anterior end of a testis, have been described by Marshall, '84, Kent, '85, and Ridgewood, '88; the reverse condition, with the ovary below the testis, has been found by Bourne, '84, in Rana temporaria. Marshall, and also Smith, '90, have reported cases in which one of the sex-glands in an individual was an ovary and the other a testis. This latter form of hermaphroditism is extremely rare among amphibians, and it has not yet been found in Bufo. Evidently hermaphroditism occurs much less frequently among the Urodela than among the Anura, as only two cases have as yet been reported for this group of amphibians. La Valette St. George, '93, has given a brief description of a case of hermaphroditism in Triton tæniatus, and Knappe, '86, has noted the presence of a Bidder's organ in a young salamander; neither investigator gives any details regarding the structure of the ovo-testes in these forms.

Abnormalities of the kind described above, whether they occur

in the sex-glands of young or of adult amphibians, seem explicable only on the assumption that the primitive amphibians were hermaphroditic, and that in the course of their evolution this primitive hermaphroditic condition has given place to the bi-sexual condition found at the present time. Although a rudimentary sex-gland (Bidder's organ) is found only among the Bufonidæ at present, traces of the primitive hermaphroditic condition still exist in other forms, as is shown by the fact that in some individuals the genital glands contain both ova and spermatozoa. Such individuals, however, are only rudimentary hermaphrodites, since apparently only one kind of germ-cell ever becomes functional. The spermatozoa are probably a much more specialized type of cell than the ova, and it might be expected, therefore, that the male germ-cells would assume the characteristics of ova more frequently than that the female germ-cells would be able to develop into spermatozoa. This is doubtless the reason that, with but very few exceptions, all of the anomalies found in the sex-glands of adult amphibians occur in individuals which must be considered as males since only the sperm-cells are able to reach maturity.

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EXPLANATION OF FIGURES.

All figures were drawn with the aid of a camera lucida. They have been reduced two-thirds.

FIGS. 1-16.—Outline drawings showing the various types of anomalies found in the genital organs of young toads killed soon after completing their metamorphosis. C.A, corpora adiposa; B.O, Bidder's organ; T, testis; O, ovary; N, kidney. × 26.

FIG. 17.—Longitudinal section through the enlargement in the lower part of the left ovary which is outlined in Fig. 10. × 250.

FIG. 18.—Transverse section through the enlargement in the lower part of the right ovary which is outlined in Fig. 2. × 250.

FIG. 19.—Section of an ovum found in the rudimentary ovary of an hermaphroditic toad. This cell has all of the characteristics of the young ova normally present in Bidder's organ. Y.N, yolk-nuclei. × 1000.

FIG. 20.—Section of the nucleus of a rudimentary ovum which is just beginning to degenerate. × 333.

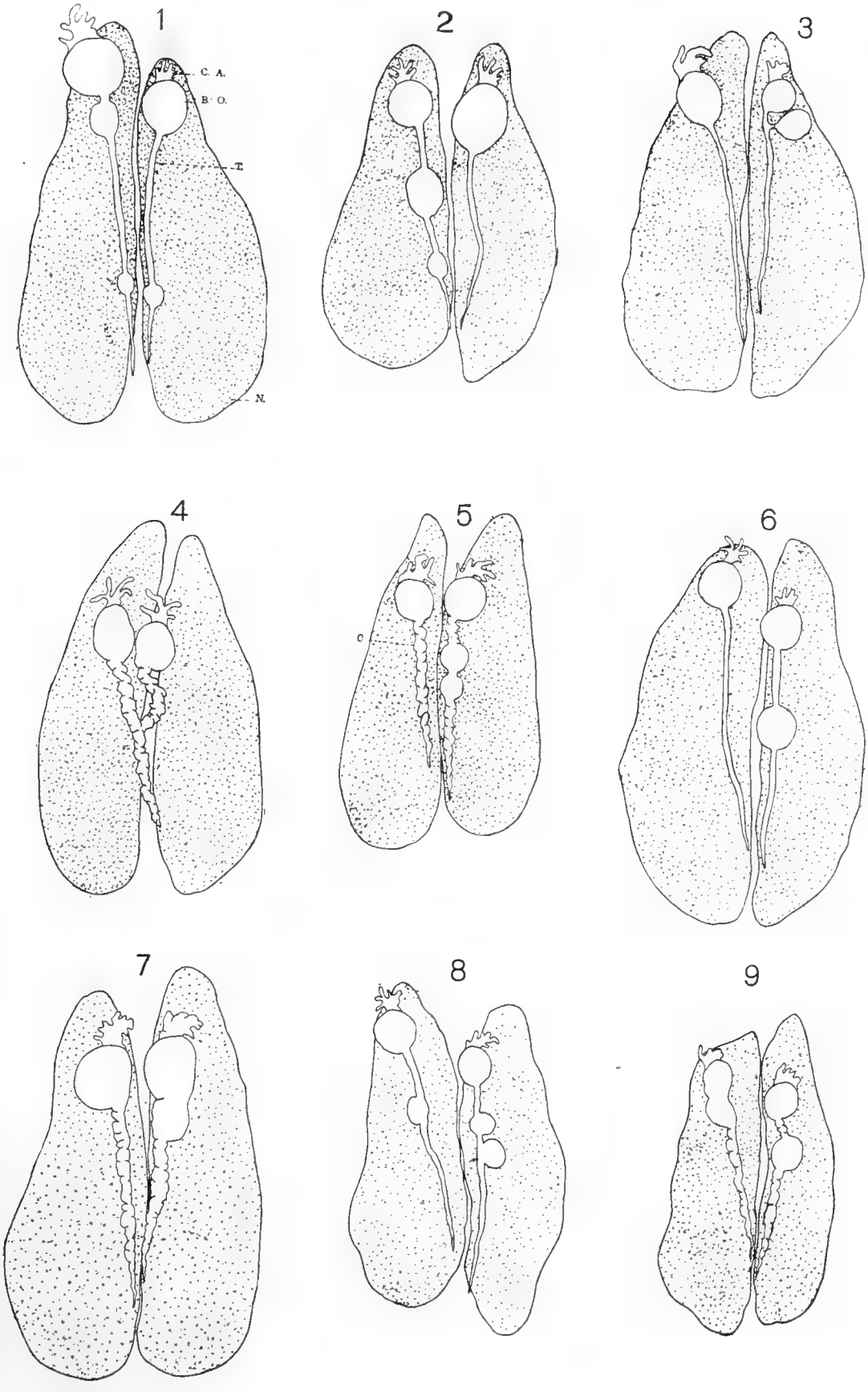
FIG. 21.—A later stage in the degeneration of the nucleus of a rudimentary ovum. × 333.

FIG. 22.—Section of the nucleus of a young oöcyte which appears normal. × 1000.

FIG. 23.—Part of a section of a large ovum showing vacuolization of the cytoplasm. × 333.

FIG. 24.—Part of a section of a large ovum having two distinct layers of yolk spherules in the cytoplasm. × 333.

FIG. 25.—Section of the nucleus of a large ovum which appears normal. × 333.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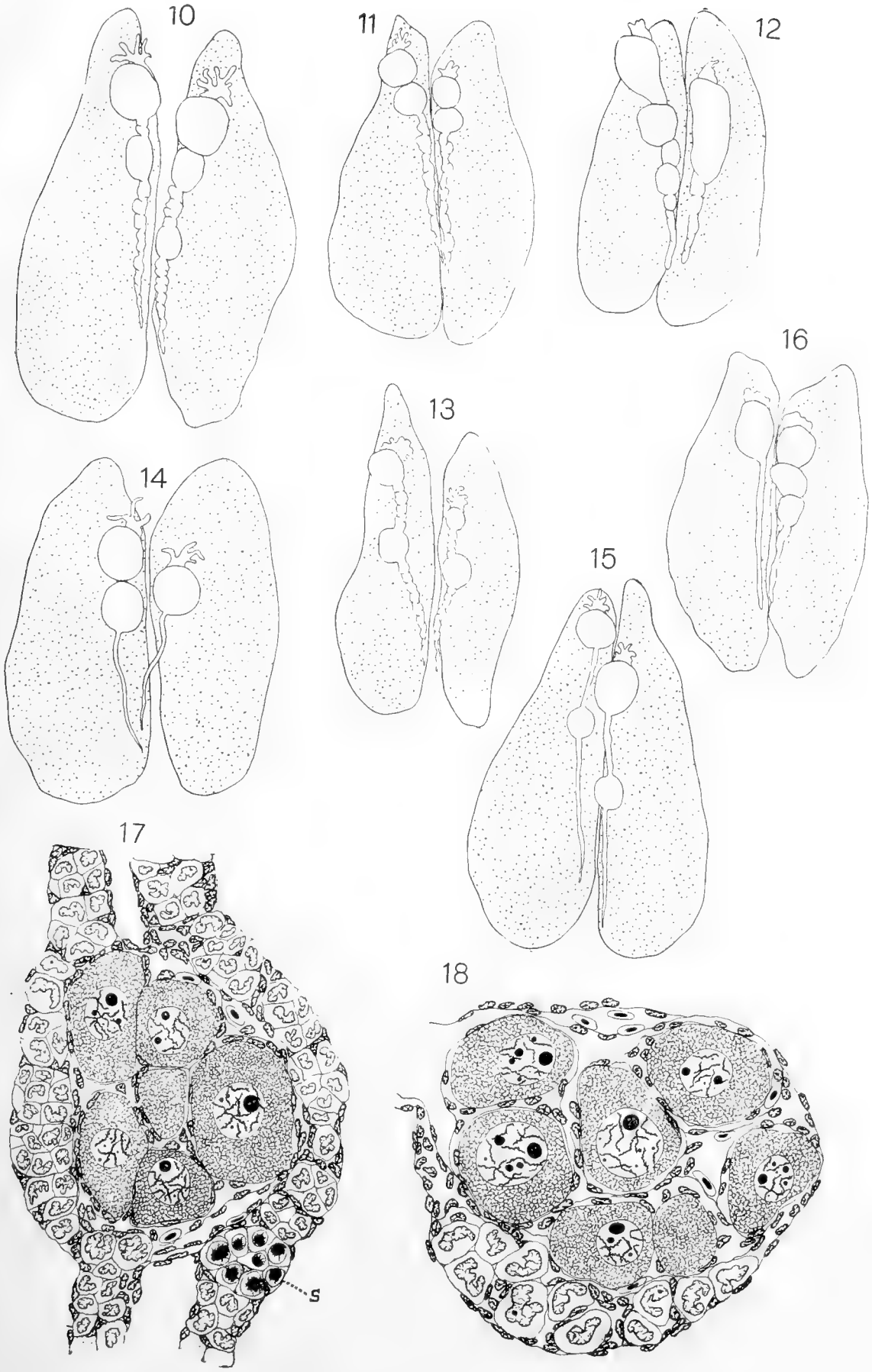
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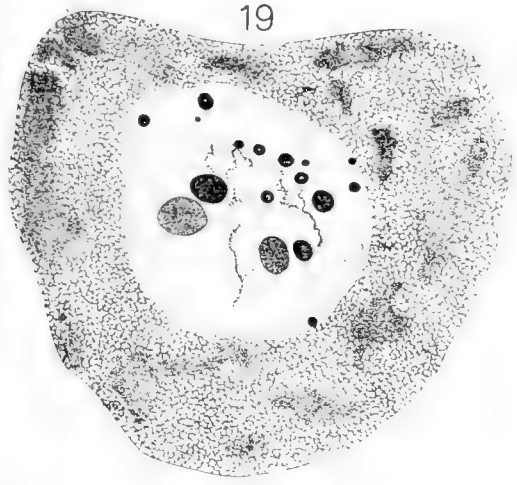

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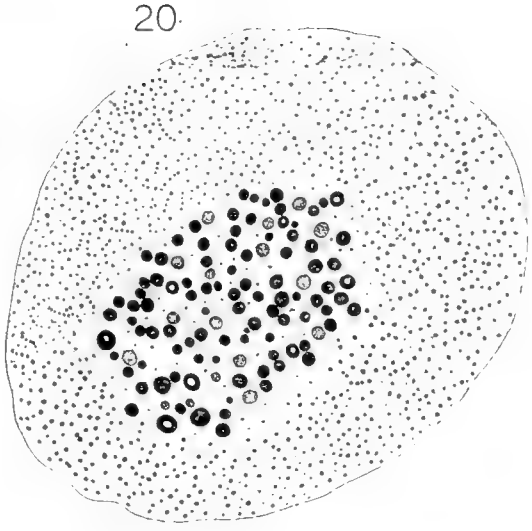

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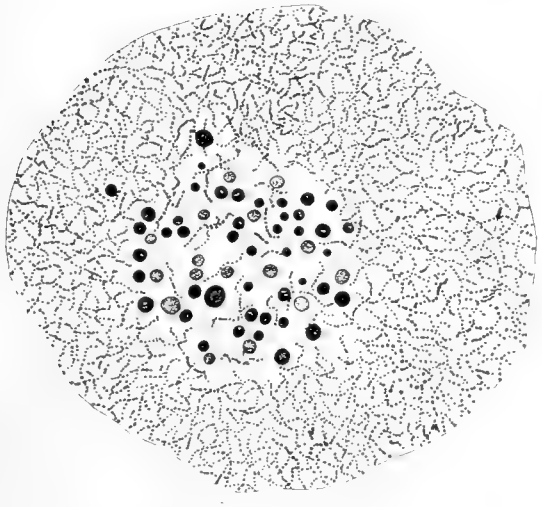

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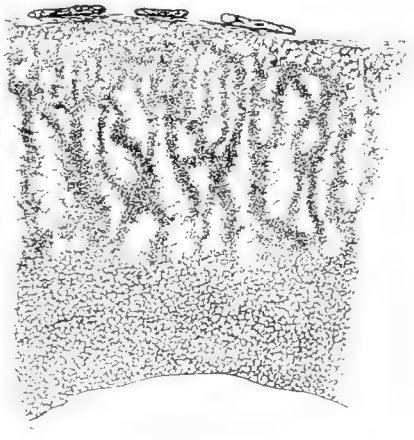

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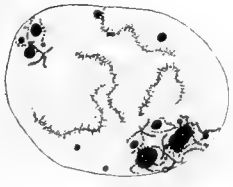

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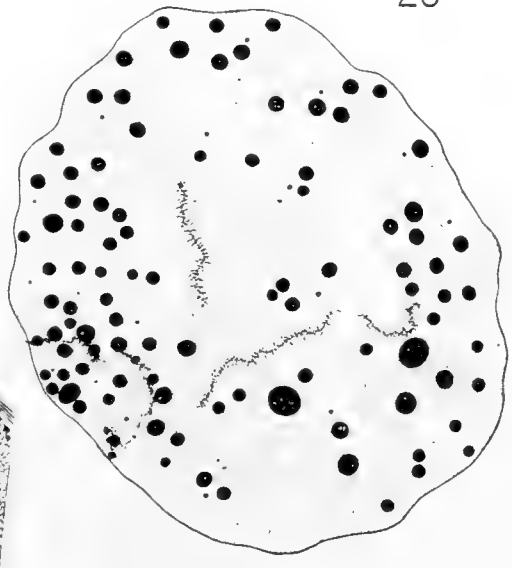

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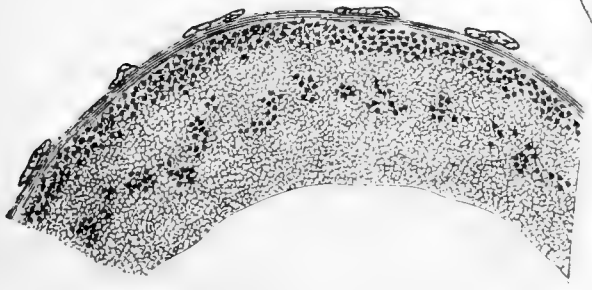

THE ANATOMY AND DEVELOPMENT OF THE JUGULAR LYMPH SACS IN THE DOMESTIC CAT (FELIS DOMESTICA).

BY

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From the Anatomical Laboratories of Columbia and Princeton Universities.

WITH 66 FIGURES.

CONTENTS.

CONTENTS—*Continued*

INTRODUCTION.

In January, 1908, the writers read a paper before the Association of American Anatomists upon the "Anatomy and Development of the Jugular Lymph Sacs in the Domestic Cat." This paper, which includes merely an outline of the development of the sacs was subsequently published in the Proceedings of the Association.[1]

In dealing with this subject in the present paper it is the intention of the writers to give a minute and detailed account of the development of these sacs in the domestic cat so that every phase and condition of their development may be represented and illustrated by a series of reconstructions, which are as accurately reproduced as their complicated character will permit. The object of presenting so detailed an account is, to place beyond the range of inference all doubt that has hitherto existed regarding the venous origin of the jugular lymph sacs, as well as to establish a basis for the study of the development of this portion of the lymphatic system in other mammalian embryos.[2]

In a more recent article on the "Cervical Veins and Lymphatics in Four Human Embryos" (Am. Jour. of Anat., vol. 9, page 38), Dr. Lewis states as follows concerning the first appearance of lymphatics in human embryos:

No lymphatics could be found in a 9.2 mm. embryo, so that the jugular lymphatics probably arise in human embryos of about 10 mm. This

[1]Huntington and McClure: The Anatomy and Development of the Jugular Lymph Sacs in the Domestic Cat. The Anatomical Record, vol. 2, nos. 1 and 2, 1908 (pages 1-18, 17 figs.).

[2]In a paper by N. W. Ingalls (The Anatomical Record, vol. 2, no. 8, 1908), dealing with the vascular system of a 4.5 mm. human embryo, certain vessels are figured which open into the post and precardinal veins near their confluence to form the duct of Cuvier. These are undoubtedly, as Ingalls suggests, veno-lymphatic anlages of the jugular lymph sac, if judged by the corresponding structures observed in embryos of the cat.

accords with the observation that they first appear in rabbits of 9.5 to 10 mm., but does not agree with Ingalls' opinion that in a 4.9 mm. human embryo certain vessels represent the first anlage, or earliest forerunners, perhaps, of the lymphatic system in man. The vessels in question are clearly veins.

Unless the conditions in man differ widely from those in the cat, it is evident to the present writers that Dr. Lewis has not as yet identified the earliest venous anlages or forerunners of the jugular lymph sac in human embryos, but has observed only the derivatives of these anlages after they have become distinctly lymphatic in character and when, by themselves, at this stage of development, they present no direct evidence that they have been derived from the veins.

In the course of our work on this subject, which has extended over a period of four years, we have been constantly surprised by the extraordinary variability and complexity which characterize the detailed development of the jugular lymph sacs, and it has only been possible to interpret these conditions after the study of a very complete series of embryos, including a number of embryos of approximately the same ages. It has also been necessary to consider, in connection with the embryonic history of the jugular lymph sacs, these structures and their variations in the adult.

The primary principles underlying the development of the jugular lymph sacs are (1) the development of a secondary channel parallel to the embryonic precardinal and the Cuvierian end of the postcardinal; (2) the association with this secondary channel of a certain number of dorsal precardinal tributaries, and (3) the separation of these two sets of venous elements, which we have termed "Veno-lymphatics," from the main venous channels and their subsequent conversion into the definite jugular lymph sacs by a process of growth and fusion.

Although a general principle of development has not been difficult to establish, it has proved, in some cases, a matter of the greatest difficulty to determine the actual mode of origin of certain of the venous anlages (veno-lymphatics) of the jugular lymph sacs owing to the variable manner in which these veno-lymphatics

develop in conjunction with the main venous channels and their tributaries, as well as the variable manner in which they fuse together. On account of the large number of reconstructions (71), which we have made of the region in which the jugular lymph sac is developed, we feel, however, that we are able to present a fairly accurate account of all stages of their development.

Our observations have been based wholly upon the study of wax reconstructions made after a slight modification of the method of Born. The method of plastic reconstruction was alone used by us for the reason that we found it the only one by means of which it was possible to get every vascular element in the reconstruction and to establish thereby a graded series of stages, complete in all details, in which the vascular elements could be studied and compared in their proper relations. Moreover, in view of recent publications on the vascular system, it is necessary to point out the self-evident fact that the only other available *extra-vitam* method of studying the development of vascular elements, viz., by injection, will, if successful, only demonstrate channels or spaces actually continuous with each other at the time of the injection, but will completely fail in revealing vascular spaces as yet independent of those injected, although subsequently a connection between the two may be established.[3]

We concluded at the beginning of our investigation that the method of injection would prove inadequate as a means of determining the actual mode of origin of the anlages of the jugular lymph sacs. That our conclusion was correct or not, the reader may best judge for himself.

Most of the embryos studied by the writers were fixed in Zenker's fluid and the sections then stained on the slide with Delafield's haematoxylin and Orange G. For fixation of tissues and differentiation of vascular structures, this method has proved most satisfactory.

Opposite is a list of the cat embryos studied and in part reconstructed by the writers in connection with this investigation.

[3]See figs. 1 and 2 in Dr. Sabin's paper on The Lymphatic System in Human Embryos. The American Journal of Anatomy, vol. 9, 1909, page 64.

LIST OF MATERIAL EXAMINED.

SOMITES	SERIES	MILLIMETERS	SERIES	MILLIMETERS	SERIES	MILLIMETERS	SERIES
11	188	8	3	12	78	16	12
13	86	8	89	12	97	16	15
		8.5	102	12	100	16	95
Millimeters	Series	8.5	75A	12	217	16	96
4.5	82	9	5	12.5	5	16	222
5	134	9	19	12.5	28	16	224
5	11	9	106	13	35	16	230
5	47	9.2	136	13	92	16.5	197
5 +	30	9.5	132	13	107	16.5	240
5 +	31	9.5	239	13.5	76	17	23
5.18	188	10	33	13.5	189	17	36
5.6	110	10	79	13.5	223	17	94
5.7	103	10	101	14	7	17	142
6	84	10	111	14	34	17	190
6	85	10	112	14	37	17.5	17
6	115	10	113	14	122	18	20
6	116	10	114	14	127	18	87
6	117	10	140	14	210	18	88
6	128	10	237	14	211	18.5	10
6	187	10.3	81	14	212	18.5	21
6.2	109	10.5	118	14	214	18.5	199
6.5	130	10.5	120	14.5	9	19	80
6.5	131	10.7	474 (Har-	14.5	38	20	83
6.5	129	vard Embryological		15	53	21	242
6.5	186	Collection).		15	53	22	16
6.8	105	11	6	15	75	25	22
7	138	11	18	15	91	25	44
7	2	11	27	15	216	25	147
7	8	11	77	15	218	31	144
7	135	11	98	15	219	34	168
7	137	11	213	15	243	35	90
7.2	108	11.5	29	15	244	37.5	99
7.2	119	12	1	15.5	141	45	14
7.2	121	12	24	15.5	143	51	104
7.25	13	12	25	15.5	215		
7.50	32	12	26	16	245		

The measurement of each embryo was made after fixation. All measurements of embryos referred to in this paper represent the distance between the crown of the head and the baseof the tail (Crown-rump measurement).

Series 1 to 74 belong to the Princeton Embryological Collection,

and from series 75 on, with the exception of series 474, to the Embryological Collection of Columbia University (College of Physicians and Surgeons).

A SUMMARY OF FORMER INVESTIGATIONS BEARING UPON THE DEVELOPMENT OF THE MAMMALIAN JUGULAR LYMPH SACS.

It is not the purpose of the present paper to give a complete historical review, nor a statement concerning the differences of opinion which exist at the present time in regard to the development of the lymphatic system in general. Both of these subjects have been fully considered by Huntington[4] and Sabin[5], and can be referred to by anyone interested in the matter.

It may be stated here, however, that all American anatomists who have thus far investigated the development of the mammalian jugular lymph sac, as distinguished from the general system of lymphatic vessels, now agree that it is derived directly from the venous system. It may, therefore, be of interest to examine the evidence upon which this conclusion was originally based. With the exception of the present writers, Sabin and Lewis, as far as we are aware, are the only investigators who have studied the development of the mammalian jugular lymph sacs.

Sabin's[6] original statement concerning the development of the lymphatic system, including the jugular lymph sacs, was as follows:

> The lymphatic system of the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction which the endothelial bud takes as it grows from the vein. In the ducts themselves there are no valves at first. From these two buds, and later from two similiar buds in the inguinal region, ducts grow toward the skin and widen out to form four sacs or lymph hearts and from these sacs the lymphatics grow to the skin and cover its surface (page 387, 1902).

[4] The Genetic Interpretation of the Development of the Mammalian Lymphatic System. The Anatomical Record, nos. 1 and 2, vol. 2, 1908, pages 19 to 45.

[5] Sabin, Florence R. Further Evidence on the Origin of the Lymphatic Endothelium from the Endothelium of the Blood-vascular System. The Anatomical Record, vol. 2, 1908.

[6] The American Journal of Anatomy, vol. 1, 1902; vol. 3, 1904 and vol. 4, 1904.

Sabin's[7] recent statement on this subject is a retraction of her former view and a complete acceptance of the work of Lewis,[8] concerning which she writes as follows:

> He (Lewis) showed that there is first a plexus of veins in the region of the sac; these veins are in free connection with the jugular vein. Then the plexus of veins is cut off from the jugular and appears full of blood, but without venous connections. Later the plexus forms a sac and rejoins the main vein. After it has joined the vein it becomes empty of blood. This discovery of Dr. Lewis I can entirely confirm.

In view of her present position, that the anlages of the lymph sacs are first cut off from and then later rejoin the veins, it appears to the writers that Sabin's original observations upon the development of the jugular lymph sacs must have been made upon embryos in which the lymph sacs had already been established, and in which the secondary permanent connections with the veins had taken place, since in all of the embryos injected she found the lymph sacs to be in open communication with the veins. If a connection between lymph sac and vein served as the only criterion for inferring that the lymph sac is derived from the veins, it appears to the writers, in view of her more recent statement on the subject, that a similar and equally valid inference might have been drawn by observing the conditions which prevail in the adult. Considering our present knowledge of their development, it is evident, from the very nature of the case, that the injection method, as used by Dr. Sabin, has not as yet given us the slightest clue as to the actual manner in which the anlages of the lymph sacs are derived from the veins, nor, in fact, any evidence at all that they even possess a venous origin.

The chief service thus far rendered by the injection method is the determination of the extent to which the lymphatic development has progressed in the body after the lymphatics themselves have been established.

[7] Loc. cit. p. 49. The Lymphatic System in Human Embryos. With a Consideration of the Morphology of the System, in The American Journal of Anatomy, vol. 9, 1909.

[8] Lewis. F. T. The Development of the Lymphatic System in Rabbits. The American Journal of Anatomy, vol. 5, 1905.

We now pass to a consideration of Lewis' investigations upon the development of the mammalian jugular lymph sacs.

Lewis[9] is unquestionably the first investigator to furnish the clue for the proper interpretation of the development of the mammalian jugular lymph sac. In the summary of his paper (p. 110) he states that the lymphatic system begins along the internal jugular vein as a detached sac formed by the coalescence of several venous outgrowths. This sac, after uniting with other independently derived lymphatics to form a continuous system, acquires a new and permanent opening into the vein near the subclavian termination.

Lewis' paper deals chiefly with a description of stages in which the jugular lymph sacs have already been formed. Only two embryos are figured and described by him in which there appears to be any evidence whatever that the lymph sac is derived from the veins, and, on the basis of these two embryos his above-mentioned conclusions concerning the development of the jugular sac were apparently formed. These are two rabbit embryos measuring 9.5 and 10 mm., respectively, in length and concerning which he writes as follows:

> In a rabbit of 13 days, 9.5 mm., no lymphatics could be found. The reconstruction, fig. 1, shows the veins along which the first lymphatics are soon to appear. The internal jugular vein receives a great many small branches. One of these, nearly parallel with the dorsal border of the vein and wider than the others, opens into the vein at either end. It is in relation with the third cervical nerve. From its position and appearance it is believed that this branch of the vein becomes a lymphatic vessel (p. 98).
>
> The second reconstruction is a 10 mm. embryo of 14 days. In this specimen a chain of lymphatic spaces has appeared along the internal jugular and the dorsal root of the primitive ulnar veins. The most anterior segment of the chain extends back to the third cervical nerve. It sends out short blind sprouts like a vein and contains many blood corpuscles. The partition between it and the jugular vein is very thin, and at one point there is a suggestion of communication between the two, as shown in the figure. No opening into the vein can be demonstrated,

[9] Loc. cit.

however. The second segment of the chain, proceeding posteriorly, extends to the fifth nerve. It equals the internal jugular vein in diameter, and is closely applied to its wall. Behind the third nerve it sends a blind diverticulum around the ventral end of the dorsal body muscles, into the deep subcutaneous tissue of the back. This diverticulum, not matched on the opposite side of the embryo, contains blood which apparently entered it from rough treatment in preserving the specimen. The third segment of the chain, between the fifth and sixth nerves, seems to connect with the root of the ulnar vein. This connection, however, lies in the plane of section, and a thin intervening wall may have been carried away in the process of cutting. A detached lymph space follows the dorsal root of the ulnar vein. A small and somewhat questionable one, not matched on the opposite side, rests against the superior vena cava between the roots of the ulnar vein. The most significant structure found in this embryo is a space filled with blood, which opens into the external jugular vein near its junction with the internal jugular. This space lies quite near the third segment of the lymphatic chain. On the opposite side of this embryo, and in the following one, this blood-filled sac connecting with the vein appears to be replaced by a lymphatic space, detached from the vein, but connecting with the chain (p. 99).

Concerning the next oldest stage he states that

Fig. 3, from an embryo of 14 days, 11 mm., shows the fusion of all the lymphatics of the previous stage into one large sac which encircles the external jugular vein. On neither side could this sac be seen to communicate with the vein (p. 99).

Upon stages older than those mentioned above he made the following observations concerning the relations of the jugular lymph sacs to the veins:

In a 14.5 mm. rabbit (14 days, 18 hours) he found that

on the right side of the embryo, in one section (No. 476), a minute orifice connected the sac and the vein. It was not in the position of the adult opening between these structures, and was not matched on the opposite side (p. 101).

In a 20-(21)mm. pig embryo he states that "no connection between the jugular sac and the veins could be detected" (p. 103).

In a 15mm. cat embryo he observed that

in one section (266) a branch of the jugular sac may enter the innominate vein a little anterior to the subclavian, but it is not clear that an actual opening exists and none can be found on the opposite side (p. 103).

Finally, referring to a 21mm. rabbit of 17 days, he states that

the jugular lymph sac on the left side, except for an extensive rupture does not connect with the vein. On the right, a pore is found leading from the sac to the internal jugular vein near its union with the external, but this also may be artificial. Thus in all the series of rabbits no bilateral communication of the lymphatics and veins, in the position of the adult openings, could be found. The pores, sometimes detected in various positions, are not adequate to empty the large sacs, and may indeed be artifacts. Communication with the veins in these must be by osmosis, therefore, and the permanent outlets of the lymphatic system must develop later (p. 109).

The above quotations include all of the data given by Lewis which bear in any way upon the actual development of the jugular lymph sacs. On examining these data it can be seen that his general statement, given in the Summary (p. 110), that the lymphatic system of rabbits (jugular lymph sac) begins along the internal jugular vein as a detached sac formed by the coalescence of several venous outgrowths, covers the general ground of lymph sac formation, but that his conclusions, while perfectly correct in their bearing on veno-lymphatic development, are based on insufficient observations. We therefore feel justified in making the statement at the beginning of our paper that our object in presenting so detailed an account is to place beyond the range of inference all doubt that has hitherto existed regarding the venous origin of the jugular lymph sacs. Although Lewis has been justified in drawing this inference, had he examined a large number of embryos of each stage, ranging between 6 and 14 mm. in length, he would have, unquestionably, been able to establish a definite venous origin for each of the segments of the lymphatic chain in the 10 mm. rabbit, without expressing any doubts whatever.

Considering the fact that we now know the jugular lymph sac to be derived from the veins, the question arises as to the develop-

mental period in which we may regard these venous derivatives as having assumed a lymphatic significance. Is it at a time when the adult condition has been reached and the lymphatic system begins to perform its destined function? Is it at a time, as in Lewis' 11 mm. rabbit (14 days) in which the venous anlages have fused into two large sacs, containing no blood corpuscles, which, in virtue of their complete separation from the main venous channels, cannot, in the generally accepted sense, function in their present condition in the lymphatic organization of the body? Is it at a time, as in Lewis' 10 mm. rabbit (14 days), in which his chain of lymphatics, constituting the jugular lymph sac, contains many blood corpuscles, and possibly communicates with the veins? Or, finally, is it at a time, as in the 8.5 mm. cat and in stages which precede it, in which the anlages of the jugular sacs are in the process of being separated from the venous channels but are still in communication with them?

The development of the jugular lymph sacs is not a haphazard process, and their anlages, although derived from the embryonic veins, are as definite in their character and mode of development as those of other organs. If we are able to draw a distinction between thymus and pharyngeal tissue at any time before the thymus anlages have separated from the pharynx and become transformed into a functional body, we are likewise justified in drawing a similar distinction between the lymphatic and venous structures. It is not the purpose of this paper to enter further into a discussion of this question of specificity of tissues, but merely to point out that the claim, made by Dr. Sabin and others, that no lymphatics make their appearance in the body before the jugular sacs are formed, can best be answered by asking for the definition of a lymphatic.

In a former contribution to this subject, the writers applied the term "Veno-lymphatics" to all the venous anlages of the jugular sacs, at a time when these anlages were filled with blood and in free communication with the venous channels. In early stages the only structural distinction that can be made between the venous anlages (veno-lymphatics) of the jugular sacs and the fully formed sacs themselves, is that the former, being in com-

munication with the veins, are filled with blood and appear to function as veins, while the latter apparently do not.

THE RETENTION OF THE EMBRYONIC JUGULAR LYMPH SAC AS AN ADULT STRUCTURE AND THE GENERAL CHARACTER OF THE LYMPHATICO-VENOUS CONNECTIONS IN THE ADULT CAT.

A detailed description of the variable conditions manifested by the lymphatics of the neck region in the adult cat is a subject upon which one of the writers (Huntington) is at present engaged.

The question concerning the fundamental character of the communications which exist between the lymphatics and the veins on each side of the body in the adult cat, as well as that concerning the persistence of the embryonic jugular lymph sac in the adult, have, however, claimed our attention.

An examination of a large number of adult cats[10] has shown that a communication between the lymphatics and the systemic veins may normally occur, on each side of the body, *at either one of two or at two typical districts* which correspond, approximately, to the angles of confluence formed by the union of the internal and external jugular[11] (common jugular angle) and of the common jugular and the subclavian veins (jugulo-subclavian angle), respectively. We have found that neither one of these two angles of venous confluence predominates as the place of communication, but that either one of the two or both may serve in this capacity, and for this reason both districts must be regarded as constituting the normal points at which in the adult cat the lymphatics communicate with the systemic veins.

The three forms of communication normally met with in the adult cat are shown in fig. 1[12] (left side) in which a communication

[10] Figs. 1, 2 and 3 are selected from a series of 180 lymphatic injections of the adult cat (Nos. 13, 26 and 40), which form the basis of a forthcoming publication on lymphatic variation in this species. They are reproduced here to define the fundamental plan of the adult connections of the jugular lymph sacs with the veins.

[11] This vein, strictly speaking, is a common jugular vein in the cat but on account of its large size, as compared with the internal jugular, is usually spoken of as the external jugular vein, of which the internal jugular is a tributary.

[12] All of the figures referred to in this paper are arranged in sequence on plates and a description of the same, preceding the plates, may be found on page 308.

is *alone* present at the *common jugular angle* (Common Jugular Tap); in fig. 1 (right side) and fig. 2. (left side), in which a communication is *alone* present at the *jugulo-subclavian angle* (Jugulo-Subclavian Tap) and in fig. 2 (right side) and fig. 3 (both sides) in which a communication is present at *both* of these angles.

As will be fully described in the following pages, the duplex character of the communication in the adult finds its explanation in the circumstance that the embryonic jugular lymph sac on each side of the body develops two caudally directed processes (Jugular and Subclavian Approaches), which are potentially capable of establishing a communication with the veins at the common jugular and jugulo-subclavian angles, respectively, and through which either one of the two or both of the typical points of communication in the adult cat are invariably established.

The jugular lymph sac of the embryo, which is, relatively, a very large structure, is in the adult much reduced in size and extremely variable in form. Figs. 2 (right side) and 3 (both sides) clearly illustrate the fact that the relations of the thyro-cervical artery to the jugular lymph sac are the same in the adult as in the embryo. This artery, a branch of the subclavian, lies ventral to the caudal end of the jugular lymph sac, both in the embryo and in the adult, and passes between the two typical points at which the jugular lymph sac communicates with the veins. *As far as we are aware the persistence of the jugular lymph sac as a distinct anatomical structure in an adult mammal has not been hitherto recognized.*

We must also protest against the assumption that the jugular lymph sacs as a whole undergo subsequent transformation into lymph-nodes. Since they receive on the one hand the main systematic lymphatic trunks of the entire body, and on the other empty into the venous system, they necessarily maintain their lumen uninterrupted. In our series of 180 adult cats they uniformly appear as distinct, more or less well-defined sacs. Their walls are definite, firmer than those of the lymphatic vessels which empty into them, somewhat thinner than the walls of the veins, and often, in fully injected preparations, multilocular or diverticular. Exactly the same conditions are presented in

the adult human subject, and the investigations of McClure and Silvester show that this arrangement is found in a very large series of mammalian forms. As far as our own observations on the cat and in man are concerned, we have drawn the following conclusions:

(1) The Saccus lymphaticus jugularis is a distinct adult mammalian structure, connected on the one hand with the systemic lymphatic trunks of the entire body, and on the other with the systemic veins, and transmitting the flow of lymph from the lymphatic into the venous system.

(2) Serving in this manner as the lymphatico-venous connecting channel, the jugular sac maintains throughout adult life its character as a receptacle of distinct and relatively large caliber, and is not extensively involved in lymph node formation.

(3) The current anatomical descriptions of the termination of the "thoracic duct" in the "jugulo-subclavian angle," or, according to variations, in the individual veins of this confluence, should be modified to correspond to the actual conditions above described.

(4) It is scarcely necessary to call attention to the surgical importance of the jugular sac in man. In cases of large and multilocular sacs the structure might be easily wounded in operations involving the jugulo-subclavian region. On the other hand, under these circumstances, it should be possible to close the wound successfully by suture of the walls of the sac, without interfering with lymphatic return through the thoracic duct and the other tributaries of the sac.

ON THE ESTABLISHMENT OF THE LYMPHATICO-VENOUS CONNECTIONS OF THE ADULT.

One of the greatest difficulties encountered in our investigation was the determination of the exact manner in which the permanent lymphatico-venous connections are established in the adult.

Do the veno-lymphatic anlages of the jugular lymph sacs undergo a complete separation from the main veins and then, after fusing to form the jugular sacs, secondarily rejoin the veins at either one of the two or at both of the points at which a communi-

cation may be found in the adult (jugulo–subclavian angle and common jugular angle), or, do the two points of communication found in the adult represent primary connections between the main venous channels and the veno-lymphatic anlages of the jugular sacs which have persisted throughout development?

There appear to be only two methods of determining these questions, namely, by the study and reconstruction of serial sections and by the injection of the venous, veno-lymphatic and lymphatic vessels in embryos of the appropriate stages. As far as our observations on embryos of *Felis domestica* are concerned, we are in the position to make the following definite statement which is based on an exhaustive study of numerous series of embryos.

In embryos between 10.7 and 14 mm., inclusive, (crown-rump measurement) a very constant and definite picture is offered, in strong contrast both with the free veno-lymphatic connections of earlier stages, and with the definite and typical taps at the two above-defined points which are encountered in the later stages and which are present, one or both, in the adult. In this intermediate condition, the caudal portion of the emptied sac appears to end blindly in two processes (the Jugular and Subclavian Approaches). These blind terminals extend to the immediate neighborhood of the common jugular and jugulo-subclavian angles, in other words, to those points at which in the earlier stages the last of the primitive connections between the venous system and the lymphatic sac persist for a time, and at which in later stages, after the establishment of the adult type, the wedge-shaped lymphatico-venous entrance with valve formation is found. But, in the intermediate stages now under consideration, the most careful scrutiny under high power, of excellently fixed and stained series in large number, fails to reveal any definite continuity whatever between the lymph sac and the vein. We have here, therefore, a positive and apparently uniform observation which, in judging the general question at issue, cannot be disregarded, since one well-established fact is of more value than any number of conjectures. This fact we regard as definitely determined, within the natural limits which are imposed by errors in observation and interpretation of a

microscopical field. We have had no personal experience with the injection method, as practised by Professor Sabin and others, but have had the opportunity of seeing at a meeting of the Anatomical Association a number of her series sectioned after injection. The chance of extravasations of the injection fluid, coupled with the further chance of injury in section-cutting, seems to us to preclude the possibility of drawing definite conclusions by this method as to the communication or non-communication of venous and lymphatic spaces. The close approach in many instances of the blind ends of the lymphatic sac to the angles of venous confluence and the delicacy of the intervening tissue, raises the possibility of a rupture occurring, to a probability, if an injection were made in either direction. However valuable the injection method may prove in determining the extent and further growth of the lymphatic system when once established, we have felt that it would not yield trustworthy results in determining the problem now under consideration. On the other hand, in view of the uncertainty of injections, it is evident that even a negative result would not be conclusive and could not be accepted as valid evidence that the lymphatic sac no longer communicated with the venous system.

We are obliged, therefore, in the present state of our knowledge, to confine ourselves to the above statement, viz., that, as far as observation can determine, an intermediate stage exists in which temporarily the lymph sac is completely separated from the venous system, with which at a later period it makes secondary connections which constitute the definite and permanent adult lymphatico-venous taps.

As bearing somewhat on this question, the conditions noted above in the adult cat (cf. p. 188) deserve further consideration. While in many individuals both the common jugular and jugulo-subclavian taps are present (fig. 3), others possess only a single adult lymphatico-venous connection on each side (fig. 1), which may be located at either one of the two typical points. We consequently deal in these latter cases with instances in which one or the other of the primitive taps (always present in the early veno-lymphatic stages) is given up during the process of development

and is not re-established. If one of the original connections is thus abandoned, it is at least evidence that both are not inviolable lymphatico-venous junctions which persist throughout development. It therefore appears quite as logical to suppose that, after temporary complete separation of the lymph sac, in the aberrant adults referred to, a secondary venous connection is established at only one of the two points of election, as to argue that one of the primitive taps persisted throughout in an unrecognizable form, while the other was entirely lost.

The view that the lymphatico-venous communications of the adult are secondarily established in the embryo was first advanced by Lewis who arrived at his conclusion on the grounds that he was unable to determine the presence of a communication on opposite sides of the embryo (rabbit and pig) in the position of the adult openings, and that in many instances no communication could be detected at all.

McClure and Silvester[13] have recently shown that the plan of communication in the adult rabbit and pig is exactly the same as that in the adult cat, in that it may occur on each side of the body, at either one of two, or at two typical districts (common jugular and jugulo-subclavian angles). Therefore, while the absence of a symmetrical arrangement of communications on both sides of the embryo is no longer proof that the communications are secondarily established, the fact that no communications have been detected at all in certain stages cannot be disregarded in favoring this view.

Following is a description of the relations observed by the writers which exist in the cat between the jugular lymph sac and the veins in embryos measuring over 10.5 mm. in length:

In a 10.7 mm. embryo (Harvard Embryological Collection, Series 474) no communication could be observed between lymph sac and vein on either side except possibly at a point situated slightly cranial to the jugulo-subclavian junction (right side, slide E, section 194, and left side, slide E, sections 197-198). These points of communication are extremely doubtful in character but

[13] McClure and Silvester. A Comparative Study of the Lymphatico-venous Connections in Adult Mammals. The Anatomical Record, vol. 3, no. 10, 1909.

are of interest on account of their bilateral arrangement as well as for the reason that they correspond approximately in their position to that of the primary connection which exists at an earlier stage between the veno-lymphatic plexus and the base of the primitive ulnar vein. If it is true that the sac separates temporarily from the venous system, then it is quite natural that pictures should be observed during the process of separation and before the same is quite completed, which would lead to the description of these "extremely doubtful" connections.

In a 11 mm. embryo (series 27) in which the jugular lymph sac was established, no communication between lymph sac and vein could be detected. Two processes of the lymph sac, however, approach and almost reach the two points at which the lymph sac was formerly connected with the veins. In a former paper we have termed these two processes the *Jugular* and *Subclavian Approaches,* respectively.

In a 12 mm. embryo (series 78) no communication could be detected on the right side. On the left side of the same embryo however, the anterior end of the lymph sac was found to be in wide-open communication with the internal jugular vein (anterior tap of evacuation) but was completely separated from the veins at all other points. This condition in an embryo of this age will be further considered in connection with another topic.

In a 13 mm. embryo no communication between lymph sac and veins could be detected on either side.

In a 14 mm. embryo examined by the writers a doubtful communication was observed on the left side, situated slightly cranial to the jugulo-subclavian junction (series 34, slide 21, section 24). No communication could be detected at the junction of the external and internal jugular veins.

In another 14 mm. embryo (series 37) a tap was established at the common jugular angle, but no connection was found at the jugulo-subclavian confluence.

In the above sense, if the final connections are secondary then, in the beginning of their establishment (14 mm.), stages might be found which give incomplete and doubtful pictures of lymphatico-venous connection when compared with the definite and typical arrangement once fully established.

In a 15 mm. embryo (series 75) the caudal end of the left jugular lymph sac (subclavian approach) was filled with blood corpuscles and communicated with the venous system in an unmistakable manner at the jugulo-subclavian junction, by means of a wide opening (slide 8, section 22, left side). A doubtful communication was also apparent at a corresponding point on the right side, but in neither case could one be detected at the junction of the external and internal jugular veins. In another 15 mm. embryo (series 53, slide 11, sections 23 and 24) the caudal end of the lymph sac was in wide-open communication with the vein on both sides of the embryo, at a point slightly in front of the jugulo-subclavian junction. These connections in the 15 mm. embryo may be secondary taps, fully established, but not yet invaginated into the typical wedge-shaped entrance, and prior to the valve formation.

In a 15.5 mm. embryo (series 141) no indication of a communication could be observed on either side. In this case either the measurement of the embryo may have been modified by variations in curvature, or the secondary connections belated.

From the 16 mm. embryo on, in which the thoracic duct usually forms a continuous vessel and communicates with the left jugular lymph sac, we have found no difficulty in determining the presence of a lymphatico-venous connection or tap at either one of the two, or at both of the points at which the lymphatic system communicates with the veins in the adult. The lymphatico-venous connection or tap met with in these more advanced embryos differs widely in character from that found in the 15 mm. embryos as well as from that found in the preceding veno-lymphatic stages, in which a wide-open communication may be present. The lymphatico-venous connection in these advanced embryos is formed at each of the two typical adult points of entry by a wedge-shaped process of the jugular lymph sac which is deeply invaginated into the angle of confluence of two veins, or into one of the veins contiguous to the point of confluence, and which opens into the vascular lumen by a narrow slit-like aperture bounded by a two-lipped valve. This type of embryonic lymphatico-venous connection was first demonstrated by the writers at the meeting of the

Association of American Anatomists in 1906[14] and is well illustrated by figs. 4, 5, 6, and 7 which are photomicrographs of transverse sections of a 16 mm. cat embryo taken at the level at which the external jugular joins the jugulo-cephalic trunk. Figs. 4, 5, 6 and 7 represent, respectively, sections 233, 237, 239 and 241 of series 15.

DEVELOPMENT OF THE VENO-LYMPHATIC PLEXUSES AND SACS OF THE EARLY EMBRYONIC STAGES AND THE ESTABLISHMENT OF THE STRUCTURAL BASIS FOR THE SUBSEQUENT DEVELOPMENT OF THE JUGULAR LYMPH SACS.

The general development of the jugular lymph sacs in the cat presents four successive periods:

1. The primary venous stage, in which the ground-plan of the primitive embryonic venous system is laid down.

These early embryonic venous channels appear to crystallize out of an antecedent capillary network along definite hydrostatic lines.

2. Veno-lymphatic stage, in which, with reduction and further definition of the venous channels, a portion of the same is surrounded in certain areas by a secondary capillary network which, together with a larger or smaller portion of the main embryonic vein, is condensed into a uniform structure and separates from the permanent venous channel as the anlage of the future jugular lymph sac.

In view of the double relation which this structure sustains, on the one hand to the embryonic venous system from which it is directly derived, and on the other to the general lymphatic system with which it establishes secondary connections, we have designated this period as the *Veno-Lymphatic Stage* in the development of the definite jugular lymph sac, and have described the portions separated from the early venous channels as the *Veno-Lymphatic Plexuses* or *Sinuses*, which form the anlage of the final definite jugular lymph sac.

3. Pre-lymphatic stage, in which the early veno-lymphatic plexus becomes sac-like and evacuates its blood-contents, and

[14] Huntington and McClure. Figs. 14 to 17 inclusive, in vol. 2 of The Anatomical Record.

then appears to become temporarily detached from the venous channels from which it arose.

4. Secondary or definite lymphatic stage, in which the evacuated sac becomes assigned to the lymphatic system, establishing, on the one hand, connections with the independently developed general systemic lymphatics, and, on the other, reëntering the permanent venous channels by apparently secondary connections made at typical points, thus forming the link which in the adult unites the definitely organized venous and lymphatic systems.

It is desirable, for reasons which will appear in the presentation of the subject, to deal with the individual problems involved on the following basis and in the order indicated:

I. General Ground-Plan of the Embryonic Venous Area Involved in the Subsequent Development of the Jugular Lymph Sacs.

II. Analysis of the Developmental Stages in the Formation of the Jugular Lymph Sacs.

III. Detailed Description of the Individual Stages.

IV. Summary and Conclusions.

I. GENERAL GROUND-PLAN OF THE EMBRYONIC VENOUS AREA INVOLVED IN THE SUBSEQUENT DEVELOPMENT OF THE JUGULAR LYMPH SACS.

It is necessary to consider in the first place the general structural ground-plan of the principal embryonic venous channels concerned in this process, and to establish certain main divisions of the same to which subsequent reference can be made in dealing with the individual series.

The portion of the embryonic venous system involved in the development of the jugular lymph sacs includes a large part of the precardinal vein, the district of its confluence with the postcardinal vein to form the duct of Cuvier, and the proximal segment of the postcardinal vein caudal to this point.

This venous area can be mapped out into the districts shown in the composite average schematic fig. 8 containing the following subdivisions:

Precardinal area

1. Arched Portion or Cephalic Arch of the Precardinal Vein
2. Caudal or Straight Segment of the Precardinal Vein

Area of Pre- and Postcardinal Confluence and of Postcardinal Veins

3. Jugular Promontory and Duct of Cuvier
4. Primitive Ulnar Segment of Postcardinal

The definite connections between the fully developed lymphatic and venous systems in later embryonic stages and in the adult fall within these districts and occupy normally two typical and, within certain limits, constant points:

1. Common jugular tap: At the angle of confluence of internal and external jugular veins.

2. Jugulo-subclavian tap: At the jugulo-subclavian angle of confluence.

Precardinal Area.

1. *The Cephalic Arch of the Precardinal Vein.*

The precardinal vein begins as a strongly curved intracephalic arch receiving a number of dorsal tributaries along its convexity and a number of branches along its concavity.

A. Dorsal Tributaries of the Cephalic Arch.

The dorsal tributaries entering the cephalic arch along its convexity vary in number and in mutual relations in individual embryos and in embryos of closely related stages.

One large tributary of this group (A–B in the schematic figs. 8 to 16) is uniformly present and in later stages forms the direct continuation of the caudal or straight segment of the precardinal vein.

In addition to this large tributary a number of smaller secondary dorsal tributaries of the cephalic arch may be encountered both cephalad and caudad of this main vessel (fig. 8, a′, b′, etc.).

These additional tributaries (**b′**, **b″**, **b‴**) caudad of the main trunk of A–B are serially more or less in direct line with the set of primary dorsal precardinal tributaries (**1**, **2**, **3** and **4** in fig. 8) entering into the subsequent formation of the veno-lymphatic plexuses. As far as our observations extend, tributaries **b′**, **b″** and **b‴** do not generally enter into the composition of these plexuses, although it is possible that in the later stages, in certain circumstances, they may be retained as secondary anterior channels of communication between the fully established veno-lymphatic sac and the precardinal vein.

The possible involvement of these tributaries (**b′**, etc.) of the cephalic arch in the late veno-lymphatic stages, in connection with the establishment of an anterior tap of evacuation is discussed in dealing with the series concerned (cf. p. 292, series 78, fig. 57).

B. Ventral Tributaries of the Cephalic Arch.

In the early stages the concavity of the cephalic arch receives a number of tributaries which temporarily drain a territory subsequently drained by the permanent external jugular vein. The vagus and spinal accessory nerves lie in close relation to these branches, and in later stages these vessels anastomose around the spinal accessory so that the nerve then penetrates the precardinal arch from the medial to the lateral aspect through an oblique foramen.

2. *Caudal or Straight Segment of the Precardinal Vein.*

The cephalic arch is continued caudad into a second nearly straight segment of fairly uniform caliber. This portion of the vein is the more important of the two, since both the dorsal circumference of the vein itself and its dorsal tributaries (**1**, **2**, **3** and **4**, in fig. 8) are involved in the subsequent development of the veno-lymphatic plexuses.

A. Dorsal Tributaries of the Caudal or Straight Segment of the Precardinal Vein.

This segment may receive four distinct and separate tributaries (**1**, **2**, **3** and **4**, fig. 8) or four distinct groups of tributaries, depend-

ing upon the developmental stage and individual variation. Of these tributaries the one labeled **4** in fig. 8 is the most important, not only on account of the extensive drainage area which it controls, both caudad and cephalad of its precardinal terminal, by means of its branches **a** and **b**, but also on account of the relations which it subsequently maintains with respect to the development of the anlages of the jugular lymph sac (caudal division of the ventral veno-lymphatic plexus).

B. Ventral Tributaries of the Caudal or Straight Segment of the Precardinal Vein.

This portion of the precardinal usually receives two main branches, frequently joined at their terminal to include a fenestra.

Area of Pre- and Postcardinal Confluence.

3. *Jugular Promontory and Duct of Cuvier.*

At the confluence of the pre- and postcardinal veins the main venous channel enlarges into a capacious swelling, which gives rise ventrally to the duct of Cuvier, while it projects dorsally as a rounded protuberance which we have designated as the *Jugular Promontory*. This promontory constitutes an important region in the future history of the veno-lymphatic and the definite lymphatic structures. Primarily it develops as the result of the confluence of large venous trunks. It receives on its ventro-lateral aspect the cephalic vein, and ventrally the anlage of the external jugular which, after confluence with the cephalic vein, forms a trunk of large size entering the ventro-lateral aspect of the promontory. The jugular promontory usually receives in later stages the three or four well-defined dorsal tributaries (**5S, 6S, 7S,** and **8S**), in fig. 8, which enter its convexity along the dorso-medial aspect. In the later stages the jugular promontory also receives dorsally the drainage from the body-wall and anterior limb-bud by the primitive ulnar vein. The development of this vein will be considered under the following topic *The Primitive Ulnar Segment of the Postcardinal Vein.*

In the early stages, the cephalic arch of the precardinal and the drainage area of its caudal or straight segment is situated lateral to the otocyst. Subsequently a network of anastomosing venous channels develops on the medial aspect of this structure and finally forms the main intracranial channel. Tributaries draining this area in part empty directly into the newly formed channel and in part pass caudad to terminate in the dorso-medial aspect of the jugular promonotory caudal to the entrance of the primitive dorsal somatic branch 4 in fig 8. This change enables the caudal or straight portion of the precardinal to give up, in the majority of cases, the dorsal tributaries **1, 2, 3,** and part or the whole of **4,** and to contribute them toward the development of the veno-lymphatic plexuses. Consequently in certain stages the straight segment of the precardinal vein may appear partially denuded of dorsal somatic tributaries. The somatic vessels from **5S** *to* **7S** or **8S,** inclusive, descending caudad, enter the dorso-medial aspect of the promontory, while the dorso-lateral part of the promontory and precardinal, cephalad of the promontory, as well as precardinal tributaries **1, 2, 3** and **4,** in whole or in part, are involved in the veno-lymphatic development.

It is thus seen that the large size of the jugular promontory is due to the number and increasing caliber of the trunks, which progressively become confluent at this point, and that it represents so to speak, the slack of the early embryonic jugular system which permits, in the subsequent cardiac descent, the drawing out of the redundant promontory into the elongated vessel of later stages.

4. *Primitive Ulnar Segment of the Postcardinal Vein.*

Caudal to the entrance of dorsal somatic tributary **6S** or **7S,** (**6S, 7S,** fig. 8) into the jugular promontory, and to the origin ventrally of the duct of Cuvier, the *postcardinal* vein of the early stages, in the area receiving the seventh or the eighth, to the twelfth dorsal somatic branch (**7S, 8S,** fig. 8), gives rise to a secondary parallel channel, the *primitive ulnar vein.* This vessel develops along the dorsal circumference of the postcardinal by

condensation of a peri-venous plexiform capillary network. The impetus to its formation is apparently given by the return circulation from the anterior limb-bud through the marginal vein. This vessel, in its rudiments, at first drains into the somatic branches of the umbilical vein. With the diversion of the umbilical to the hepatic circulation and with the resulting transferal of the somatic return to the postcardinal line, the primitive ulnar appears as a secondary derivative from the latter, serving, before the establishment of the subclavian vein, as the main channel returning the blood from the anterior extremity. As such, it develops from the capillary network surrounding the postcardinal vein by condensation of the plexiform reticulum along the dorsal circumference of the main vessel, and opens into the promontory at the level of the sixth dorsal somatic tributary (**6S** in series 106, 9 mm. embryo, fig. 40).

The series of fenestræ in the postcardinal vein (fig. 8), leading up to the jugular promontory, indicate the line along which subsequently the primitive ulnar vein, by further extension and confluence of the fenestræ, continues to separate from the postcardinal vein proper, until only its cephalic termination into the dorsolateral aspect of the jugular promontory is retained as its definite point of connection with the embryonic venous system.

The part played by this vessel in the early drainage of the anterior limb-bud, in the subsequent development of the dorsal veno-lymphatic plexus, and its final relation to the adult type of lymphatic organization, makes it one of the most important factors in the evolution of the definite lymphatic system. The detailed history of this vessel may therefore be properly considered under the separate headings, dealing with the individual stages.

II. ANALYSIS OF DEVELOPMENTAL STAGES IN THE FORMATION OF THE JUGULAR LYMPH SACS.

As already stated, the jugular lymph sac, or anterior lymph heart, develops in the embryo of the Domestic Cat as a direct derivative of the early embryonic venous system. It arises from certain of the dorsal tributaries of the precardinal and from the

main channel of the pre- and postcardinal veins, adjacent to and including their point of confluence to form the duct of Cuvier. Two distinct ontogenetic types are involved in the production of this structure:

1. A certain number of dorsal somatic tributaries of the early precardinal vein become dilated and fuse together, retaining at first their primary connections with the main vein. This process in the mammalian embryo, preliminary to definite lymphatic formation, is strictly homologous to the development of the lymph hearts in the lower vertebrates. Sala's[15] researches on the development of the posterior lymph heart in the embryo of the common fowl prove that this organ is developed entirely by dilatation and fusion of the lateral branches of the first five coccygeal veins.

In the mammalian embryo, and specifically in embryos of *Felis domestica*, the first four dorsal somatic branches of the straight or caudal segment of the precardinal vein are, to a greater or less extent, involved in the formation of the jugular lymph sac, the homologue of one of the lymph hearts of lower vertebrates.

2. Thus, while in mammals this phylogenetic type of lymph heart formation is preserved, it plays but a secondary part in the production of the jugular lymph sac. The greater part of this structure, which in certain embryonic stages reaches a relatively enormous development, as compared with its condition in the adult, is directly derived from the main systemic veins by a process, which we have called "*fenestration.*" By this designation we do not mean to define a method of histogenetic formation of the early embryonic blood vessels, but to accentuate, by the use of a brief and serviceable term, the fact that in certain stages in the development of the venous system, stages of the greatest importance in preparation for the appearance of the veno-lymphatic or lymph heart division of the lymphatic system, the early embry-

[15] Sala, Luigi, Ricerche fatte nel laboratorio de Anatomia Normale della R. Universita di Roma: vol. 7, pp. 263-269, April, 1900.

onic pathways exhibit a typical and characteristic picture, best defined by the term "fenestration." The main lines of venous drainage have already become fairly defined out of the antecedent primary capillary network from which they arise. In the further growth of the embryonic tissues secondary plexiform capillaries surround the main vessel and become connected with the same. By condensation of this plexus and enlargement of the interreticular spaces the principal embryonic veins appear in this developmental phase as channels perforated by larger spaces or "fenestræ" within the general confines of the main trunk. In a subsequent stage confluence of these "fenestræ" results in the more or less complete separation of the primary vein into two parallel secondary channels, which appears to be a uniform principle in the development of parallel venous trunks. Or, as in the case of the jugular lymph sac, further extension of this same process may result in separating from the main venous channel elements which unite to form a closed sac entirely distinct from the vein from which it arose.

The term "fenestration" means, therefore, in the sense in which it is employed in this paper, one of the *last* stages in the definite crystallization of the venous system out of an indefinite antecedent plexiform condition, and the determination of an important element in lymphatic organization, closely associated with the embryonic venous system. It does *not* mean the formation of lacunæ or spaces in veins already fully established, but is used by us as a convenient and short term to define the conditions actually and frequently encountered in reconstructions of the veno-lymphatic period. Thus the description of a portion of the early venous area "separating by fenestration" from the remainder to form a discrete and separate element is to be interpreted as indicating in a few words the more complicated processes just outlined. Such "fenestral separation" may result in the establishment of a secondary vein paralleling the course of the main channel, as in case of the posteardinal and primitive ulnar veins, or, specifically, in the development of the jugular lymph sacs the areas involved "separate by fenestration" from the vein proper to constitute the anlages of that portion of the lymphatic system

which is derived, as a veno-lymphatic plexus or sac, or modified mammalian lymph heart, directly from the veins. Some of the general problems involved in the question of the early embryonic venous organization have been recently considered by Clark,[16] Evans,[17] and by Schulte and Tilney,[18] while the mechanical basis of early embryonic vascular organization forms the subject of a classical monograph by Thoma.[19]

To return to the analysis of the development of the jugular lymph sac we therefore find that along the dorso-lateral circumference of the early pre- and postcardinal veins, in the appropriate stages, the redundant embryonic venous channels appear perforated by a linear series of openings or fenestræ. At first these fenestræ are small, but subsequently they enlarge, elongate and then adjacent fenestræ become confluent.

In this manner a segment of the originally uniform pre- and postcardinal veins gradually becomes separated from the dorsal circumference of the parent vein, and forms a secondary parallel "para-precardinal" or "para-postcardinal" channel which, together with the above mentioned precardinal tributaries, constitutes the anlage of the jugular lymph sac. To these venous derivatives of the precardinal tributaries and of the main channels we have, as above mentioned, given the name "veno-lymphatics."

One important condition develops as the result of this method of formation, namely, the progressive increase, up to a certain point, in the number of the communications which may obtain between the veno-lymphatic anlages of the jugular sac and the systemic veins. At first these communications in the precardinal region are represented by the terminals of the three or four dorsal

[16] Clark, Elliott R: Observations on Living Growing Lymphatics in the Tail of the Frog Larva. The Anatomical Record, vol. 3, 1909.

[17] Evans, H. M. On the Earliest Bloodvessels in the Anterior Limb Buds of Birds and their Relation to the Primary Subclavian Artery. The American Journal of Anatomy, vol. 9, no. 2, pp. 281-319, May, 1909.

[18] H. von W. Schulte and Frederick Tilney: A Note on the Organization of the Venous Return, with Special Reference to the Iliac Veins. The Anatomical Record, vol. 3, no. 11, 1909.

[19] Thoma, R. Untersuchungen über die Histogenese und Histomechanik des Gefässsystems, Stuttgart, 1893.

precardinal tributaries. The process of fenestration not only involves the portions of the precardinal vein intermediate between the terminals of its dorsal somatic branches (**1, 2, 3** and **4,** in fig. 8.), but also invades the terminals themselves (fig. 10) which usually appear enlarged and dilated in a funnel-shaped fashion at their points of union with the main trunk.

With the more marked separation of the veno-lymphatic plexuses from the main venous channels the interfenestral spaces become drawn out into elongated channels which function as numerous secondary avenues of communication between the anlages of the jugular sac and the systemic veins.

The process of secondary capillary perivenous formation leading to ultimate fenestration may first involve the main precardinal vein and then extend into the dorsal tributaries of the vein, or it may begin in the funnel shaped terminals of the tributaries themselves, and subsequently progress in them to such an extent that the original single dorsal tributary becomes split up, or separated into two or more components, depending upon the type of the plexiform and fenestral development (fig. 11).

This separation of the dorsal tributaries of the precardinal vein into a number of components is a fundamental process of veno-lymphatic development, since certain of the elements, thus secondarily derived, constitute, in part, the veno-lymphatic anlages of the jugular lymph sac, while others retain the original character of the early dorsal somatic tributaries, prior to veno-lymphatic organization, and form the series of dorsal precardinal branches, for the most part temporary, which lie in line with those opening further caudad into the promontory and the postcardinal vein.

Prior to the detailed consideration of the development of the jugular lymph sacs it is advisable to determine definitely the status of the dorsal precardinal tributaries and their share in the development of the veno-lymphatic anlages of the jugular lymph sacs.

Although the general principles underlying the development of the jugular lymph sacs are the same for all embryos, the range of individual variation is considerable. No two embryos of approximately the same measurement, in the series examined by

us, agree in every detail, and even the same embryo often shows variation and different degrees of development on opposite sides.

In the description of the individual series these special differences will be taken up in detail, but prior to their consideration it is desirable to establish the general conditions and the genetic principles involved in veno-lymphatic development.

A great part of the observed variability depends upon the fact that the jugular lymph sac of the later stages (fig. 17) is preceded by the formation of three primary veno-lymphatic plexuses (fig. 12). Subsequently these unite to form the complete jugular lymph sac (figs. 12 to 17).

In this process two of these plexuses first unite to form a common ventral division of the sac, which then fuses with the third plexus placed dorsally, to constitute the common jugular lymph sac of the later embryonic stages and of the adult.

We have consequently adopted the following descriptive terminology:

I. Ventral Veno-Lymphatic Plexus.
   A. Cephalic or Anterior Division.
   B. Caudal or Posterior Division.
II. Dorsal Veno-Lymphatic Plexus.

These three veno-lymphatic plexuses are of primary importance and represent a developmental stage intermediate between the purely venous and early veno-lymphatic conditions, and the subsequent period, in which the veno-lymphatics have fused to form a common sac. (cf. figs. 12 and 46).

For clearness of description we will divide the account of the development of the jugular lymph sac into two periods:

I. From the Development of the Early Venous Stage up to the Establishment of the Three Primary Veno-Lymphatic Plexuses.
   A. Development of the Ventral Veno-Lymphatic Plexus.
      1. Cephalic or Anterior Division.
      2. Caudal or Posterior Division.
   B. Development of the Dorsal Veno-lymphatic Plexus.

II. From the Establishment of the Three Primary Veno-Lymphatic Plexuses or Sinuses to the Attainment of the Adult Condition of the Jugular Lymph Sacs.

FIRST PERIOD.

2. From the Early Venous Stages to the Establishment of the Three Primary Veno-Lymphatic Plexuses.

A. *Development of the Ventral Veno-Lymphatic Plexuses.*

In some instances the ventral veno-lymphatic plexus is largely derived from the primary dorsal somatic branches **1**, **2**, **3** and **4**, while in others the main portion of the plexus differentiates by plexiform condensation from the precardinal vein, carrying the tributaries with it, in which case these latter play a secondary part in the development of the plexus. Finally in a third group both somatic and main precardinal components share nearly equally in the formation of the plexus. Again one portion of the plexus may be mainly precardinal in derivation, another largely composed of elements contributed by the somatic tributaries.

Hence the ventral veno-lymphatic plexus, in both of its subsidiary parts, exhibits a considerable range in variation, as will appear in the detailed description of the individual series, and it becomes necessary to analyze the two developmental processes in detail in order to prepare the way for the consideration of the conditions presented by any given case.

Moreover, in this general plan of development of the ventral plexus, dorsal somatic precardinal tributary **4** and the associated segment of the main precardinal vein take a share approximately equalling that contributed by the remaining three (anterior) tributaries (**1**, **2** and **3**) and by the portion of the precardinal vein included within their area. This results in the establishment of the two primary divisions of the ventral veno-lymphatic plexus, viz:

1. *A cephalic or anterior division.*
2. *A caudal or posterior division.*

Thus, while the genetic principles involved in the formation of the two divisions of the ventral veno-lymphatic plexus are identical, it will prove more serviceable to distinguish between the two in the preliminary analysis, because certain special features in the relation of dorsal somatic tributary **4** to the precardinal vein and the promontory modify the picture of the development of the caudal portion of the ventral veno-lymphatic plexus and serve to distinguish it in early stages from the cephalic division of the same.

We will therefore first consider the development of the cephalic or anterior division of the ventral veno-lymphatic plexus, then that of the caudal division, thirdly that of the dorsal veno-lymphatic plexus, and finally take up the mutual relations of these three divisions prior to their fusion into the common veno-lymphatic sac.

This can best be accomplished by following the veno-lymphatic development on the hand of the series of diagrams shown in figs. 8 to 13 which, with the exception of fig. 11A, represent in schema lateral views of the reconstructions of the left side in profile, but which answer equally well for the conditions found on the right side. Fig. 11A represents a dorsal view of fig. 11 in the region of the jugular promontory.

1. Development of the Cephalic or Anterior Division of the Ventral Veno-Lymphatic Plexus.

The formation of this portion of the ventral veno-lymphatic plexus can be analyzed by considering the two main types of development which it exhibits, with the understanding that in individual instances one or the other of these types may predominate, or that they may share equally in the production of this portion of the ventral plexus.

First Mode of Development. In this type the cephalic part of the ventral veno-lymphatic plexus is largely developed from the three anterior dorsal somatic branches of the precardinal vein.

The early precardinal tributaries **1, 2,** and **3** in fig. 8, enlarge at their terminals in a funnel-shaped manner, and these expanded bases present a more or less distinct plexiform or fenestrated appearance (**1, 2,** and **3** in fig. 10).

By enlargement of these fenestræ or by confluence of adjacent inter-reticular spaces, the terminals of the primary tributaries divide into two components, a lateral veno-lymphatic (**VL**) and a medial somatic (**S**) element (fig. 11).

The lateral elements, (**1VL**, **2VL**, **3VL**, in fig. 11) shift to the dorso-lateral circumference of the precardinal vein, the medial elements (**1S**, **2S**, **3S**, in fig. 11), conversely enter the dorso-medial aspect of the main channel.

At first these two sets of components are still connected by transverse channels (figs. 11 and 18).

The best example of this stage encountered in our collection is represented by Series 138 (cf. figs. 33, 34, 35 and 36 and the diagrams, figs. 18 and 37).

Subsequently these tranverse connections are given up and then the dorso-lateral elements enlarge and constitute the cephalic division of the ventral veno-lymphatic plexus (**1VL, 2VL, 3VL,** in figs. 12 and 19), while the dorso-medial branches (**1S, 2S, 3S** in figs. 11, 12 and 19) continue to function for a time as dorsal somatic tributaries of the precardinal, and are not further involved in veno-lymphatic development.

After the dorsal somatic branches (**1S**, **2S**, and **3S**) have separated completely from the veno-lymphatic components (**1VL**, **2VL**, **3VL** in figs. 12 and 19), the latter may or may not fuse with each other in the early veno-lymphatic stages. Usually, however, the veno-lymphatic components of tributaries **1** and **2** (**1VL, 2VL,** in fig. 13), or of **2** and **3** (**2VL**, **3VL**, in fig. 12) or of **1**, **2** and **3** (**1VL**, **2VL**, **3VL**, in fig. 20) unite with each other so that their individuality is lost and they then present a more sac-like appearance than in the preceding stages.

As a result of this fusion one or two of the originally separate veno-lymphatic components give up their primary connection with the precardinal vein (fig. 13 and 21). All of these variations are not shown in the diagrams but can easily be followed in the figures of the individual reconstructions.

Typical instances of the separation of the dorsal tributaries into their veno-lymphatic and dorsal somatic components are afforded by series 138, (figs. 33-37), while the three primary divi-

sions of the veno-lymphatic plexus are well shown in series 102, fig. 46.

Second Mode of Development. In this form the dorso-lateral circumference of the straight segment of the precardinal, in the area included between the levels of the first and third dorsal somatic tributaries (**1, 2** and **3,** in fig. 8), retains the plexiform condition and by continued growth and enlargement of the interreticular spaces forms a multi-fenestrated appendage to the main venous channel (cephalic division of ventral veno-lymphatic plexus in fig. 9). By confluence of these fenestral spaces this appendage is separated as a veno-lymphatic sac from the definite vein except at its cephalic end, where one or two of the primary connections between the veno-lymphatic structure thus formed and the precardinal vein proper persist until a later stage, serving as channels for the evacuation of the blood from the veno-lymphatic sac into the definite venous system, after the three primary veno-lymphatic plexuses have fused to form a common sac. The tributaries, **1, 2** and **3** (**1, 2** and **3,** in fig. 9) appear to be taken up as a whole in this veno-lymphatic development and to lose their individual character.

2. Development of the Caudal or Posterior Division of the Ventral Veno-Lymphatic Plexus.

This portion of the ventral veno-lymphatic plexus is also formed in one of two ways:

1. From part of primary dorsal tributary **4** (**4**, fig. 8), combined with a portion of the precardinal vein which, between the early terminal of **4** and the promontory, crystallizes out of the plexiform network of the primary vein along its dorso-lateral circumference as a "para-precardinal channel" (figs. 8, 9 and 10).

2. From the para-precardinal channel alone. In this case tributary 4 is not involved directly in the veno-lymphatic process and remains in its entirety as a dorsal somatic branch secondarily entering the promontory.

In the early venous stages (fig. 8), as mentioned above, the fourth dorsal tributary of the precardinal (**4**) is by far the largest

and the most variable in form and composition of all the dorsal branches of this vein. It is formed by the confluence of two trunks (*a* and *b*) which, cephalad and caudad, follow the dorso-lateral border of the neural tube, and it opens into the precardinal by an enlarged and expanded base, frequently fenestrated, which receives a large single cephalic tributary (**4S** in fig. 8).

Precardinal tributary **4** is on the border line dividing the series of the preceding branches **1**, **2**, and **3**, uniformly involved to a greater or less extent in the development of the cephalic division of the ventral veno-lymphatic plexus, from the series of permanent dorsal somatic tributaries of the promontory (**5S, 6S,** and **7S,** in fig. 8). Consequently, while **4** shares with the anterior branches **1, 2,** and **3,** the tendency to divide by enlargement of the plexiform terminal into a medial somatic and lateral veno-lymphatic component, the tributary, as a whole, inclines in individual cases in one or the other of these directions, i. e., either toward the more complete veno-lymphatic or the dorsal somatic type of development. The genetic characters of **4** are more marked, compared with the preceding branches **1, 2,** and **3,** by reason of its large size and extensive drainage area. Certain instances, or rather certain developmental stages (cf. series 138, figs. 35, 37, 18 and 11A), offer striking pictures of the separation into a dorso-lateral veno-lymphatic (**4VL**) and a dorso-medial somatic component (**4S**), connected by a number of transverse anastomoses.

Further, in the same sense, the entire caudal division of the ventral veno-lymphatic plexus is interposed between the cephalic division of the same and the dorsal veno-lymphatic plexus, which is developed entirely from the promontory by condensation of the plexiform dorso-lateral circumference of this segment of the main venous channel, without involving the promontorial dorsal somatic branches (**5S, 6S,** etc.).

Hence the caudal division of the ventral veno-lymphatic plexus of the early stages inclines in some instances toward the developmental type of its cephalic associate, i. e., it involves dorsal somatic tributary **4** to a greater or less extent. In other cases it follows more closely in its development the succeeding dorsal veno-lymphatic plexus, i. e., it is derived largely or entirely from

the circumference of the main vein, by further extension of the para-precardinal channel, while in a third group both the precardinal vein and tributary 4 share more or less equally in furnishing the elements which enter into the composition of the caudal division of the ventral veno-lymphatic plexus.

Consequently an analysis of primary somatic tributary 4 and of the related precardinal segment, in reference to veno-lymphatic development, will give the clue to the interpretation of the various conditions encountered in the detailed study of the individual series.

In the early stages the blood current of tributary 4 (4 in fig. 8), empties by a dilated terminal into the straight segment of the precardinal.

Subsequently, a secondary channel (para-precardinal channel) is separated by condensation of plexiform areas of the main vein from the latter between the terminal of 4 and the promontory. This secondary "para-precardinal channel" (figs. 9 and 10) now drains the blood current of tributary 4, originally emptying into the precardinal, for the greater part directly into the promontory. The dilated and frequently fenestrated terminal of 4 and the para-precardinal channel, secondarily segregated from the main precardinal line, undergo veno-lymphatic development and form the structural basis for the further growth of the caudal division of the ventral veno-lymphatic plexus.

In this process as stated above, tributary 4 may be largely taken up in the veno-lymphatic organization, or the latter may chiefly involve the main precardinal channel, leaving 4 to maintain its somatic character.

Hence in general the caudal division of the ventral veno-lymphatic plexus presents the two main genetic types:

1. *Derivation from the para-precardinal channel and the veno-lymphatic component of* 4, *leaving the dorsal somatic element of* 4 *as the first of the promontorial dorsal somatic branches.*
2. *Derivation entirely from the para-precardinal channel, without involving any portion of tributary* 4, *which*

remains intact as the first somatic promontorial tributary and does not participate in veno-lymphatic development.

The two main genetic types observed by us in the development of the caudal division of the ventral veno-lymphatic plexus, in embryos between 7 and 10 mm., may be presented schematically in the series of diagrams figs. 8 to 14 (lateral views, left side) and in figs. 11A, 18 and 19 (dorsal views).

1. *Deriviation from para-precardinal channel and veno-lymphatic component of precardinal tributary* **4**.

Early Venous Stage (fig. 8).

Tributary **4** of the precardinal series is marked by its large size and extensive area of drainage, and by its expanded plexiform terminal.

Intermediate Stage.

The beginning of veno-lymphatic organization in general is indicated along the line of the primary dorsal precardinal tributaries by expansion and reticular fenestration of their terminals. In the area controlled by precardinal tributary **4**, the precardinal begins to differentiate along its dorsal circumference a secondary para-precardinal channel (figs. 9 and 10).

Early Veno-lymphatic Stage.

The precardinal tributaries, in general, have begun to separate into dorso-medial somatic (**1S, 2S, 3S,** and **4S**), and dorso-lateral veno-lymphatic components (**1VL, 2VL, 3VL,** and **4VL,** in figs. 11, 11A and 18).

The para-precardinal channel, having, for the most part, assumed the function of draining the territory formerly controlled by the primary precardinal terminal of tributary **4** (fig. 8), has become associated with the veno-lymphatic component of **4** (**4VL**), which in turn is in process of multiple fenestral separation from the somatic element of **4** (**4S**). Cf. fig. 11A which is a dorsal view of fig. 11 in the region of the promontorio-precardinal angle and fig. 18, also a dorsal view, which shows this process of separation at a more advanced stage of development.

Later Veno-lymphatic Stage (figs. 12, 13, 14 and 19).

The separation of the veno-lymphatic (lateral) and somatic (medial) components of tributary **4** has been completed. Each component now obtains a separate and independent entrance into the main venous channel.

The para-precardinal channel has further separated from the main vein and has fused with the veno-lymphatic element of tributary **4** (**4VL**) into a single sac (figs. 13, 14 and 19), to form the caudal division of the ventral veno-lymphatic plexus which is now no longer connected by transverse anastomoses with the somatic portion (**4S**) of tributary **4**.

The above outlined plan of development for the caudal division of the ventral veno-lymphatic plexus, appears to be more or less uniform in character for cat embryos, in general, in which the para-precardinal channel and tributary **4** combined, enter into the formation of this plexus.

2. *Derivation entirely from the para-precardinal channel, without involving any portion of tributary* **4**.

This condition is illustrated by series 2, fig. 32, and by the diagram fig. 19.

In this case the caudal division of the ventral veno-lymphatic plexus appears to be developed entirely by further growth and enlargement of the para-precardinal channel, without involving dorsal somatic tributary **4** which secondarily enters the promontory independently of the para-precardinal channel, as the first of the series of dorso-medial branches draining into this segment of the venous system.

Here the caudal division of the ventral veno-lymphatic plexus follows absolutely the developmental path of the dorsal veno-lymphatic plexus.

In connection with these two main genetic types of development two conditions have been observed by us which are worthy of special mention.

(*a.*) In one case (fig. 20, diagram) the veno-lymphatic (**4VL**) and somatic (**4S**) elements of tributary **4** separate completely by

fenestration in the usual manner. The latter (**4S**) then enters the promontory as its first dorsal somatic branch. The former (**4VL**) joins the veno-lymphatic para-precardinal channel, enlarging the caudal division of the ventral veno-lymphatic plexus and very probably forming in part or in whole the rounded appendage of the same noted in some of the later stages (fig. 14 and series 77, figs. 51 and 52).

(*b.*) In another case (fig. 21, diagram), the caudal division of the ventral veno-lymphatic plexus joins the cephalic division and retains its early connections with the precardinal vein. The para-precardinal channel separates from the promontory at its caudal extremity, leaving the original point of connection with the main vein as a prominent conical teat or projection, lateral and close to the entrance of **4S** into the promontory.

It is of course evident that in an analysis of this kind it is practically impossible to distinguish *always* between clearly defined *types* of development and *stages* fixed in individual embryos which may or may not represent partial approximations to a single common genetic ground-plan. In other words, it is impossible, in studying any given series, to foretell the exact details of further development which this particular embryo would have exhibited if allowed to continue its course.

On the other hand, we feel justified in establishing the above indicated gradations of the veno-lymphatic development by reason of the very large number of embryos which we have critically examined. We believe that this exhaustive study has enabled us to present a concise composite account of veno-lymphatic development to which each series subsequently described in detail can be correctly referred, so that the unity and consistency of the fundamental plan underlying veno-lymphatic development in the cat becomes apparent.

B. The Development of the Dorsal Veno-Lymphatic Plexus

This third component of the general veno-lymphatic plexus is developed entirely from the main venous channels of the promontory and postcardinal, without involving the dorsal somatic tributaries (**5S**, **6S**, etc.), of this area.

The dorso-lateral circumference of the promontory and postcardinal carries a plexiform net work which, along the lines above indicated (page 205), condenses by fusion and enlargement of the interreticular spaces into a dorso-lateral component of the main channel, representing the anlage of the dorsal veno-lymphatic plexus. The result of this process is shown in figs. 8, 9 and 10, in which the dorsal veno-lymphatic plexus appears as a dilated sac, extending cephalo-laterad from the promontory over the junction with the precardinal and separated from the main systemic channel by a linear series of fenestræ. This plexus subsequently develops into the dorsal division of the general veno-lymphatic plexus, and forms, in later stages, after amalgamation of the veno-lymphatic components into the single jugular lymph sac, the portion of the latter to which we have applied the term "subclavian approach."

Figs. 8, 9, 10, 11 and 12 illustrate the general character and progressive growth of the dorsal plexus in the early veno-lymphatic stages, and fig. 12 the drawing out of the interfenestral areas into elongated channels, preparatory to the separation of the dorsal plexus from the promontory.

The secondary capillary network in which the dorsal plexus takes its origin from the main venous channel, extends caudad along the promontory and postcardinal vein beyond the confines of the dorsal veno-lymphatic plexus proper.

Thus the linear series of fenestræ shown in figs. 9 and 10, by enlargement and confluence, finally separate a secondary channel from the main postcardinal vein, which retains its cephalic connection with the promontory near the entrance of the sixth or seventh dorsal somatic tributaries (6S, and 7S, in figs. 11, 12 and 13). This secondary parallel vessel is the primitive ulnar vein, serving as a temporary drainage channel for the anterior limb-bud. Subsequently, in repetition of its own genesis from the postcardinal vein, a plexiform capillary network, extending along the dorsal circumference of the primitive ulnar vein, condenses into a parallel vessel which terminates in the caudal part of the dorsal veno-lymphatic plexus. Hence we have in many of our diagrams designated the area involved as the "anlage common to the primitive ulnar vein and the primitive ulnar veno-lymphatic."

In later stages this primitive ulnar veno-lymphatic becomes secondarily connected with the systemic lymphatic vessels of the anterior extremity, in the same way in which on a larger scale, the thoracic duct secondarily taps the process of the jugular lymph sac which forms its portal of entry into the same. The partial separation of the common anlage of these two structures, viz., primitive ulnar vein and primitive ulnar veno-lymphatic, is shown in fig. 12. Their subsequent development can be followed in figs. 13 to 17 incl.

SECOND PERIOD.

II. From the Establishment of the Three Primary Veno-Lymphatic Plexuses or Sinuses to the Attainment of the Adult Condition of the Jugular Lymph Sacs.

This period is characterized by the following well-marked characters:

1. Further reduction of the multiple early connections between the veno-lymphatic plexuses and the permanent veins, and the consequent more complete separation of the former from the latter, the plexuses or sac-like structures assuming a greater degree of independence. This general process of separation and loss of early communicating channels appears to proceed from both extremities of the area toward the jugular promontory where the embryonic connections are longest retained and where the two permanent adult communications between the lymphatics and the veins are established. It is to be noted, however, that in many observed cases the termination of one or more of the anterior dorsal somatic tributaries persists as an open communication between the cephalic division of the ventral veno-lymphatic plexus and the anterior part of the precardinal, and in later stages even enlarges considerably, forming the main veno-lymphatic-venous connection and functioning as the anterior tap of evacuation (cf. figs. 12 to 15, and series 78, fig. 57).

2. The three primary veno-lymphatic plexuses begin to fuse with each other to form a common sac.

Figs. 12 and 13 indicate diagrammatically the origin of the veno-lymphatic components of the jugular lymph sac above described in detail, and their partial fusion to form the single jugular sac of the later stages.

In the succeeding stage (figs. 14 and 15) the veno-lymphatic plexus further condenses by amalgamation and fusion of the primary dorsal and ventral divisions, and the communications with the main venous channels are reduced typically to three:

(1) Tap A (figs. 14 and 15). The Anterior Tap of Evacuation.

(2) Tap B (figs. 14 and 15). At the Jugulo-promontorial Angle, the future common jugular confluence, i. e., junction of internal jugular vein (precardinal) with common trunk formed by union of external jugular and cephalic veins.

(3) Tap C (figs 14 and 15). At the site of the connection of the primitive Ulnar Vein with the promontory, corresponding, approximately, to the future jugulo-subclavian junction.

The veno-lymphatic plexus as a whole is filled with blood and the plexiform character of the capillary network from which it arose is still manifested by the multiplicity of the fenestral spaces. On transverse section the walls of the original vessels entering into its composition still appear as partitions or septa which divide the interior of the sac into a complicated system of intercommunicating channels. Later, these septa become greatly reduced in number and extent so that there results a capacious sac lined smoothly by an endothelial layer, continuous with the intima of the veins.

This sac subsequently becomes emptied, the contents being evacuated through the connections still persisting with the general venous system (fig. 15, Taps A, B and C). This process of evacuation occurs with great rapidity, so that in embryos of the appropriate stages (10.5 to 12 mm.), it frequently happens that the sac on one side is completely emptied of its blood-contents, while on the opposite side it is still well filled with blood. The evacuation occurs normally at the above mentioned points, where the veno-lymphatic sac retains longest its connection with the venous system. On account of the small openings by means of which the veno-lymphatic sac usually communicates with the veins at Taps B and C, we have reason for believing that, in the majority of

cases, the sac empties itself chiefly, if not entirely, through one of the anterior connections (one or two of the primary dorsal somatic tributaries) into the cephalic end of the straight segment of the precardinal, or even that a secondary connection is established between the anterior end of the veno-lymphatic sac and the posterior extremity of the cephalic arch (through **b**,[1] etc., fig. 8), for the purpose of serving as a portal through which the blood-contents of the sac are poured into the permanent venous channels. (Compare p. 290, description of series 78, 12m).

We have designated the connections thus longest retained between the veno-lymphatic sac and the veins, as the "*taps of evacuation*," because, after the sac is fully formed, they serve the purpose of draining the blood contained in the sac, during the earlier periods, into the permanent venous channels, so that after completion of this process the sac appears entirely empty of blood, lined by endothelium, continuous through the taps of evacuation, with the intimal lining of the large veins.

As the process of evacuation takes place with great rapidity there is some difficulty in ascertaining, in individual cases, the exact point at which it occurs. The taps of evacuation apparently enlarge very much at this time, and, when the process is completed, as rapidly close. It is therefore necessary to examine numerous stages during this very short and evanescent period in order to establish the actual conditions.

After the evacuation is completed the now fully organized lymph sac separates, in the cat embryo, apparently completely for a short time from the adjacent veins, by breaking away at the evacuating point or points (fig. 16). This closed sac subsequently establishes two sets of secondary connections (fig. 17):

(*a*) With the independently formed systemic lymphatics.
(*b*) With the venous system.

These latter connections are normally formed at the two points at which the primitive promontorial connections are, in the majority of instances, longest retained, viz., at the common jugular confluence (common jugular permanent tap) and the jugulo-subclavian angle (jugulo-subclavian permanent tap, fig. 17, and

figs. 1, 2, and 3). The latter may be double, with a larger dorsal and a smaller ventral connection. The embryonic arrangement tallies with the conditions observed in the adult, in which either the common jugular, or the jugulo-subclavian tap, or both, function in individual cases as the chief portal of adult lymphatico-venous connection.

After the secondary connections with the venous system have become established, the jugular lymph sac decreases relatively in size, while the systemic lymphatic channels increase proportionately in extent and complexity. In the adult, therefore, the jugular sac appears as a greatly reduced remnant of the extensive embryonic sac and serves merely as the connecting link between the systemic lymphatics and the venous system. In a few instances portions of the early structure become invaded by adenoid growth and are thus partially transformed into lymph nodes. The lumen of the jugular sac is, however, even in these cases maintained, since it forms the channel of adult lymphatico-venous entry.

The significance of the jugular lymph sac of the mammalian embryo and the homology existing between it and the lymph heart formation in lower vertebrates has been considered in a separate communication[20] by one of the writers.

Finally, a word concerning the relations of the thyro-cervical artery and the first six spinal nerves to the veno-lymphatic plexuses and the fully established jugular lymph sac.

The thyro-cervical artery at first passes forward from its subclavian origin along the dorsal surface of the main venous channel to a point in front of the venous arch formed by the primitive ulnar vein as it enters the dorso-lateral circumference of the promontory. Here the artery turns abruptly laterad on to the lateral surface of the promontory where it divides into branches (figs. 13). In the later veno-lymphatic and lymphatic stages (figs. 14 to 17), the thyro-cervical artery still maintains

[20] G. S. Huntington, "The Genetic Interpretation of the Development of the Mammalian Lymphatic System," The Anatomical Record. Vol. 2, nos. 1 and 2, pp. 19-45, 1908.

similar relations to the main venous channel and the jugular lymph sac, lying ventral to that portion of the latter which is derived from the dorsal veno-lymphatic plexus.

In the course of its development, a large foramen is formed in the jugular lymph sac through which the first four spinal nerves pass (*SP.N.I-IV*, figs. 15 and 16). The stages which lead up to the establishment of this foramen are shown in figs. **13**, **14** and **15**. At a later stage, the dorsal and ventral portions of the jugular sac bounding the foramen, separate anteriorly and then connect secondarily with the systemic lymphatics which are formed independently of the jugular sac (fig. 17) and not, as claimed by Sabin, as the result of a centrifugal growth of the same.

The fifth spinal nerve (*SP.N.V*, in figs 13. to 17) also penetrates the jugular sac, but through a separate foramen. This condition is retained until a late stage of development.

The sixth spinal nerve (*SP.N.VI*) does not penetrate the veno-lymphatic plexus nor the jugular sac at any time. From its origin in the spinal cord it at first passes ventro-laterad between the primitive ulnar and postcardinal veins and contiguous to the point where the primitive ulnar vein arches ventrad to open into the promontory (figs. 13, 14 and 15). After the primitive ulnar vein has given rise to the primitive ulnar lymphatic and has lost its connection with the promontory, the sixth spinal nerve then passes ventro-laterad and ventral to the primitive ulnar lymphatic (figs. 16 and 17).

III. Detailed Description of the Individual Stages.

General Considerations.

The jugular lymph sacs of the cat present a considerable range of variation both in the adult structure and in the details of their development. This variability appears not only in different embryos from the same litter, and possessing approximately the same measurements, but even upon opposite sides of the same embryo, just as in the adult the right and left sides frequently show marked differences in composition and relations in the same individual. Moreover, the individual embryos differ greatly in

a chronological sense. In some, one portion of the veno-lymphatic system is well developed, while the remainder is retarded. Ultimately, within certain limits, all embryos develop congruent conditions, but owing to this variability as to the coincidence of all the factors at a given period, it has been found necessary to examine a number of embryos of about the same age, and to group the vessels thus obtained in certain cases into a composite picture which will serve as a guide for the proper interpretation of each individual embryo falling within a given period, although all of the developmental possibilities of this period may not be presented by each and every individual embryo in the series. Such a series of composite pictures has already been discussed in connection with the preceding topic (figs. 8 to 21 inclusive.)

Of course, in addition to this general source of variability, individual embryos present a certain range of personal and accidental variation in development. Not only will individual embryos show advanced development in certain areas, and retarded conditions in others, but they also differ in the minor details, such as in the number and arrangement of secondary tributaries and their anastomotic conditions.

In order to facilitate the interpretation of the individual reconstructions figured in this paper, the reader is advised to refer each one to the series of diagrams already described in the preceding pages (figs. 8 to 21, incl.).

In the figures of the individual reconstructions the approximate derivation of the components of the jugular lymph sac is indicated by the same color scheme as that adopted in the introductory analysis for the diagrams, figs. 8 to 21, inclusive, so that comparison of any given series with the corresponding generalized developmental stage described in the analysis can readily be made.

We have adopted this plan in order to emphasize the *uniform genetic type* of lymph sac development in an extensive series of embryos presenting the inevitable *individual* variations. Of course the value assigned by us in this presentation to the individual components in a given series is a question of personal interpretation of the actual conditions offered by the embryo. This

interpretation is, however, based on a vastly larger number of embryos than those figured in this paper. Each of the series quoted in our list of material has been carefully and completely examined by one or both of the authors, and many reconstructions have been made in addition to those here published. We consequently feel that the tentative value given by us to the individual developmental components of the jugular lymph sac in the embryos of the cat is based on sufficiently extensive observation and comparison, and that we are warranted in offering an ontogenetic history of the jugular lymph sac in which definite embryonic stages follow each other with remarkable uniformity in the entire series and lead uninterruptedly to the conditions established in the adult.

On account of the very narrow limits of the venous system within which the development of the jugular lymph sacs is confined, it was found advantageous, in reconstructing certain stages, to elongate the reconstruction by using thicker wax plates than the μ-measurement and magnification of the sections actually called for. This was done with the purpose of separating, and thus more easily determining, the arrangement and position of the complex of veno-lymphatic components of the jugular sacs.

With these few exceptions, all of the reconstructions shown in this paper have been reconstructed and drawn to scale. The few that have not, however, illustrate equally well the main principles of veno-lymphatic development, but appear slightly more elongated than the actual dimensions call for.

In the early stages the precardinal vein is, in conformity with the more elongated form of the embryo, less curved in its anterior or arched segment than in the later stages, and less distinctly divided into the typical districts shown in the composite scheme (fig. 8). The caudal or straight portion of the precardinal is somewhat elongated, and presents, at more or less regular intervals, oval or spindle-shaped enlargements (series 30 and 31, figs. 22 to 24), which correspond to the confluence of ventral and dorsal tributaries with the main channel. These enlargements of the precardinal are evanescent and are evidently produced by the increased amount of blood entering the main vein at these points

through the tributary branches, repeating, on a small and serial scale, the phenomena observed in the development of the jugular promontory at the site of the principal venous confluence of this region, the promontory representing in its inception a corresponding enlargement of the main vein, as yet indistinctly indicated, at the junction of pre- and postcardinal veins to form the duct of Cuvier.

These early stages offer a characteristic irregular and redundant appearance of the embryonic vein channels, which in part are but poorly differentiated against the surrounding tissue, a condition likewise characteristic of the ventral and dorsal tributaries of the main vein. In place of the more clearly defined series of collateral branches of the succeeding stages, the earlier embryos offer a number of short and more or less irregular branches, which, in their aggregate, represent the single more fully formed tributary of the later stages.

This appearance is due to the numerous secondary capillary vessels which develop around both the main vein and its tributaries, and which subsequently give rise, as above stated, to the fenestrated character of the channels, and to the appearance of the veno-lymphatic anlages.

In the following description of the dorsal tributaries of the caudal or straight segment of the precardinal, the assignment of a complex of several branches to the valuation of one of the single tributaries of later stages is based primarily upon the latter's relation to the localized enlargements of the precardinal into which the tributaries of earlier stages open. The increased vascularity of the tissues, and the resulting augmentation of the number of capillary vessels found in the area of each of the principal tributaries, is one of the phenomena preceding and directly leading up to the fenestral process above described as active in producing the veno-lymphatic condition. This secondary capillary reticulum to a certain extent masks the individuality of the primary precardinal tributaries. We have evidence that a localized group or complex of tributaries which opens dorsally into the precardinal vein in early stages may be represented by a single well-defined branch in later stages. We also have positive evidence that this

single tributary may become secondarily surrounded by, and involved in, a capillary network. We are therefore justified in assuming that the formation of a reticular complex in connection with the individual precardinal tributaries is a normal developmental character, leading up to the typical fenestral condition of the veno-lymphatic stage, and that its *establishment may occur very early or be somewhat retarded.*

This view certainly coincides with the multiple arrangement of the first three dorsal precardinal tributaries on the left side of series 30 (**1, 2** and **3,** fig. 22), and series 31 (**1, 2** and **3,** fig. 24) which are represented on the right side of series 30 by three distinct and single tributaries (**1, 2** and **3,** fig. 23).

Early Venous Stages.

Series 30, 5+$^{mm.}$ Embryo
Reconstruction of left side,
Lateral aspect, fig. 22

The cephalic or arched portion of the precardinal is moderately curved. The branches *A–B* are large and in line with the straight precardinal segment, entering the arch at its confluence with the latter. Double fenestration occurs at the junction of *A–B* with the main vein, and cephalad of the arch are several detached vascular islands. The proximal precardinal spindle **I** (**I**, fig. 22), at the posterior extremity of the cephalic arch, is only moderately developed.

The first three dorsal branches of the straight segment of the precardinal are represented on the left side of the embryo partly by single vessels and partly by plexiform capillaries. Tributary **1** (**1,** fig. 22) is a single vessel, which opens into the precardinal close to the terminal of tributary *A–B*. Tributary **2** is formed by three components (**2, 2′** and **2″**, fig. 22).

The spindle-shaped enlargements *II* and *III* in the course of the straight precardinal trunk, receiving dorsally the terminals of **1** and **2,** are, like these, closely approximated to each other.

Dorsal tributary **3** is represented on the left side of this embryo by three small branches, entering the dorsal aspect of precardinal

spindle *IV*, and by a detached vascular island not distinctly joined to the main vein, or to the tributary complex to which it belongs.

Dorsal tributary 4 (4, fig. 22) is large and typically developed. It is formed by the confluence of two extended paraneural channels (a and b) converging from the cephalic and caudal directions. Their confluence already exhibits the irregular dilatations characteristic of veno-lymphatic formation. The main right-angled trunk receives in addition a cephalic branch (4S), which represents the element of precardinal tributary 4 so often found in subsequent stages entering the promontorio-precardinal junction on its medial aspect as the first of the series of promontorial dorsal somatic tributaries (4S, figs. 11 and 12).

The precardinal spindle V is only moderately indicated. On the other hand, the distal enlargement VI is well marked, representing the anlage of the future jugular promontory. It marks the confluence of the pre- and postcardinal veins, which are still in a direct line, and gives origin ventrally to the duct of Cuvier, receiving just cephalad of this point a short branch of considerable size which probably represents the rudiment of the definite external jugular vein.

The dorsal region of this area is most interesting and suggestive of the later developmental stages. By the formation of a large fenestral space in the dorsal portion of the postcardinal, opposite to the Cuvierian confluence, an irregular dilated venous plexus (H) has been produced which projects from the vein in the area of the future promontory. This plexus represents the anlage of the dorsal veno-lymphatic plexus.

Later, with the further development of the promontory and the resulting dorso-ventral arching of the postcardinal, the dorsal veno-lymphatic fenestrated area gains the level of the promontory and rides on its cephalo-dorsal aspect.

Cephalad of this main fenestral arch H a second smaller arch (K) is developed in a similar manner from the dorsal circumference of the precardinal. This arch (K) probably represents, in part, the capillary plexus involved in the formation of the para-precardinal channel of later stages (fig. 9).

Series 30, 5+$^{mm.}$ Embryo
Reconstruction of right side,
Lateral aspect, fig. 23

A—*B*, as the main dorsal branches of the cephalic arch, occupy relatively the same position as on the left side. They are reversed in size, *A* representing the main drainage channel, while on the left side *B* is the larger of the two vessels.

The anterior precardinal spindle *I* (fig. 23, I) is well marked. A small fenestra in the dorsal portion of spindle *I* may represent the beginning separation of the element B^1, which on the left side forms a dilated appendage to *B*.

Dorsal tributaries **1**, **2** and **3** are single and distinctly defined. **1** and **2**, as on the left side, are closely approximated, so that precardinal spindles *I* and *II* are practically confluent. Precardinal spindle *IV* is well developed. It receives dorsally tributary **3**.

Spindle *V* is not pronounced, the dilatation at this point being taken up by the marked enlargement of the terminal of dorsal tributary **4**. The broad quadrilateral channel formed by the confluence of its cephalic and caudal tributaries is fenestrated at the junction with the precardinal so that the tributary enters the main vein by an anterior and posterior trunk. This fenestra represents the space framed by the venous arch which **4** in the later veno-lymphatic stages so frequently forms at its junction with the main vein, and its presence denotes the beginning formation of a reticular and fenestrated complex by means of which, as above defined, this tributary may become connected with the promontory in the later stages. From our reconstruction of these later stages it is evident that its transferal to the promontory may take place in one of two ways:

(*a*) The anterior of the two trunks forming the terminal of **4** may retain its connection with the precardinal vein, and the posterior trunk may travel back, along the line of the fenestrated reticulum, to the promontory, in which case the arch frames dorsally a vertical parajugular foramen (figs. 27, 30 and 31) or, (*b*) both trunks may become thus secondarily drained into the promontory, in which case a closed venous arch projects forward from the anterior face of the promontory (fig. 38).

There is no indication of the presence of the dorsal somatic element **4S**, which is present on the left side of this embryo (fig. 22). The reconstruction suggests that in this instance, as frequently happens, the primitive dorsal tributary **4** becomes involved in its entire extent in the development of the caudal division of the ventral veno-lymphatic plexus.

Caudad of tributary **4** the elongated distal segment of the precardinal receives dorsally three small branches (**K**) which may represent the element **K** of the opposite side, (fig. 22).

Spindle shaped dilatation *VI* is bent sharply ventrad and receives two large ventral branches, which represent the anlage of the permanent external jugular vein. As a matter of fact these vessels now open into the cardinal end of the duct of Cuvier, but with the caudal extension of the promontory, which is not yet fully established in this embryo, they will be included in later stages within the promontorial area. (See fig. 8, which illustrates diagrammatically the progressive caudal extension of the promontory and the gradual inclusion within the same of the terminals of the external jugular vein).

Dorsally, the postcardinal, at its confluence with the precardinal channel is dilated, and perforated by irregular fenestrae of varying size.

Comparison with the left side (fig. 22) suggests that confluence of these separate openings would establish the single large fenestra there encountered, in which case the channel H^1 (fig. 23) would form the main postcardinal path, while the remaining dorsal fenestrated arch would yield a para-cardinal venous arch **H** which constitutes the anlage of the dorsal veno-lymphatic plexus.

The two sides of this embryo control and supplement each other extremely well and present a typical picture of the stage concerned.

Series 31, 5+$^{mm.}$ Embryo
Reconstruction of left side,
Lateral aspect, fig. 24

The cephalic arch is somewhat more decidedly ante-flexed, and better differentiated against the succeeding straight segment of

the precardinal (fig, 24), and tributaries *A–B* are more distinctly assigned to its caudal portion than in series 30. The ventral tributaries of spindle *I* are relatively small, but more numerous.

Spindles *I* and *II* are practically confluent. Spindle *II* receives dorsally the well developed trunk of dorsal somatic tributary **1** (**1**, fig. 24), formed by the confluence of two branches, and, further caudad a secondary element, **1**[1], belonging evidently to the same drainage area, and representing the branch intermediate between **1** and **2** in series 30 (fig. 22).

There is a well marked constricted interval of the precardinal caudad of spindle *II*, between it and spindle *III*. The latter receives dorsally four branches, representing the drainage of the area assigned to dorsal tributary 2 (**2** in fig. 24).

Another elongated constricted precardinal segment intervenes between spindles *III* and *IV*. Spindle *IV* receives along its dorsal circumference six individual branches representing the drainage of dorsal tributary **3**, in the condition of multiple small elements entering the precardinal separately (3 in fig. 24).

Spindle *V* is occupied dorsally by the broad and complex union with dorsal tributary **4**. This tributary is typically dilated near its junction with the main precardinal vein, and contains two fenestral openings, which by confluence would establish the single space seen on the right side in embryo **30** (fig. 23) at the entrance of the expanded terminal of tributary **4** into the precardinal.

Owing to imperfections in the series, the embryo was not reconstructed caudal to this point. In general, compared with series 30, the embryo represents a slightly earlier developmental phase, marked by the larger number of individual elements composing the complex of dorsal tributaries **1**, **2**, and **3**, and by the presence of small intermediate tributaries between the main districts.

The general ground-plan of the embryonic venous system shown in the two series just considered (series 30 and 31) affords the most convenient approach to the detailed study of the subsequent stages in venous and veno-lymphatic development. Before proceeding to this study, it may be of advantage to consider briefly the steps by means of which the venous conditions in

series 30 and 31 have become established. They are apparently derived from the antecedent type shown in series 134 and 47. These are the earliest stages which we have completely reconstructed, and they show the building up of the primitive main systematic venous system by the confluence of at first three and then of four principal channels which unite to form the duct of Cuvier and open through the same into the sinus venosus.

Series 134, 5^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 25

In series 134 the duct of Cuvier is formed through the confluence of the precardinal, the postcardinal, the omphalo-mesenteric and the umbilical veins.

The omphalo-mesenteric vein, receiving a number of small tributaries from the pre-aortic region, possibly subcardinal radicles, arches dorso-ventrad and joins the precardinal to form the duct of Cuvier. The proximal dilated end of the omphalo-mesenteric receives on its lateral aspect the terminal of the umbilical vein. The latter is a vessel of large size, receiving by numerous tributaries the drainage from the body walls and the anterior limb bud. Along its dorsal circumference it is irregularly dilated and fenestrated.

The precardinal forms a short, strongly curved arch, receiving anteriorly several short tributaries. Near its posterior extremity it receives dorsally two very large irregularly dilated branches (4 in fig. 25). Comparison with the succeeding stages (series 30 and 31, figs. 22 to 24) suggests that they represent the elements of precardinal tributary 4.

The postcardinal vein begins anteriorly in a dilated and fenestrated extremity (*H*) which closely approaches the large dorsal tributaries of the precardinal but does not communicate with them. Near its anterior extremity the postcardinal, by a ventrally directed branch (*L*), establishes its connection at the cardinal-Cuvierian junction. Along the rest of its course it follows closely

the dorsolateral aspect of the umbilical vein and receives a number of serially arranged branches, which open into it dorsally. Caudal to the last of these tributaries indicated in the figure the postcardinal appears dilated and fenestrated and then rapidly diminishes in caliber.

Series 47, 5^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 26

The junction of the pre- and postcardinal veins is, relatively, of narrow caliber and perforated by a fenestra, agreeing with the peculiar slender and irregular formation exhibited by the Cuvierian confluence in series 134 and 30, figs. 25, 22 and 23. The omphalo-mesenteric and umbilical veins have shifted their terminals caudad and now obtain an opening into the sinus venosus independently of the duct of Cuvier proper. A branch from the body wall entering the umbilical at the sinus venosus seems to correspond to the primitive drainage of the external jugular territory. The umbilical vein also receives further caudad a number of somatic tributaries and branches from the anterior limb bud. This drainage area, compared with series 134 (fig. 25) appears reduced and in the process of transferal to the path of the postcardinal whose somatic branches are more fully developed.

The greatest interest attaches to the neighborhood of the pre- and postcardinal confluence. In both embryos (**134** and **47**) conditions are presented in this region which directly lead up to those found in the succeeding stages (embryos **30** and **31**). Two points are here involved:

(*a*) *Precardinal*

The dorsal precardinal tributary 4 (**4** in fig. 26), which is destined to play so important a rôle in the subsequent venous and veno-lymphatic development, appears to be laid down at a very early stage. In series 134 (**4**, fig. 25), it is represented by the two large dilated and tortuous branches which enter the dorsal cir-

cumference of the caudal part of the precardinal. In series 47 (**4**, fig. 26), the precardinal end of this element appears as the large single trunk entering the precardinal in the same situation. The formation of tributary **4**, with its cephalic and caudal tributary **a** and **b**, in series 30 (figs. 22 and 23), has already been considered in detail, and corresponds evidently to a further development in these later embryos of the conditions presented by the earlier series.

(*b*) *Postcardinal*

The redundant plexiform area of the postcardinal (**H**) corresponding to the dorsal aspect of the Cuvierian junction in series 30 and 31, and representing the early anlage of the dorsal veno-lymphatic plexus is already well indicated in both of the earlier stages. In series 134 (fig. 25) it is represented by the dilated and fenestrated anterior blind end of the postcardinal (*H*). In series 47 (fig. 26), it appears as the fenestrated and plexiform appendage of the dorsal circumference of the postcardinal (*H*) just caudal to the latter's entrance into the duct of Cuvier.

INTERMEDIATE EARLY STAGES OF PRE- AND POSTCARDINAL VENO-LYMPHATIC DEVELOPMENT

Series 109, 6.2^{mm} Embryo
Series 2, 7^{mm} Embryo
Series 138, 7^{mm} Embryo
Series 13, 7.25^{mm} Embryo
Figs. 27 to 39

A. General Considerations

The main venous trunks have now begun to assume the arrangement typical of the later stages.

The cephalic arched segment, conforming to the increased cranial flexure of the embryo, has turned ventro-caudad, presenting a well-defined convexity, which receives the most anterior dorsal tributaries (*A*-*B*).

The straight precardinal segment, shortened anteriorly by the

development of the cranial bend, is further condensed and appears relatively shortened by the further development of the jugular promontory.

The dorsal tributaries of the precardinal are condensed, and approximated to each other. The more numerous individual branches of the earlier stages are replaced by larger single trunks representing the three typical anterior sets, tributaries **1, 2,** and **3.**

Dorsal tributary **4** of the earlier stages now terminates, for the most part, in the promontory at the promontorio-precardinal junction. It (**4**) may be in part or entirely taken up in the subsequent veno-lymphatic formation, or it may be permanently retained as a somatic branch, in which case it forms the first of a series of somatic tributaries entering into the dorso-medial aspect of the promontory, or, finally, it may exhibit distinctly both veno-lymphatic and dorsal somatic characters, with numerous transverse anastomoses between the two elements.

In fact, all of the fundamental processes involved in veno-lymphatic development, as described above under Analysis of Developmental Stages in the Formation of the Jugular Lymph Sacs, are represented in one or the other of the above mentioned series, and may be summed up as follows:

(1.) Progressive enlargement and caudal extension of the jugular promontory. (2) Increase in secondary capillary formation and resulting increase in the reticulated and fenestrated dorso-lateral circumference of the promontory and the postcardinal vein, and consequently an enlargement of the dorsal veno-lymphatic plexus. (3) The establishment of the anlage common to the primitive ulnar vein and the primitive ulnar veno-lymphatic. (4) The transferal of the main blood current in tributary **4** from the precardinal vein to the promontory through the secondarily formed para-precardinal channel. (5) Separation of precardinal tributary **4** and its associated para-precardinal channel into a veno-lymphatic and a dorsal somatic component. (6) Separation of the precardinal tributaries **1, 2** and **3** into their veno-lymphatic and dorsal somatic components. (7) Establishment of the cephalic and caudal divisions of the ventral veno-lymphatic plexus.

B. Detailed Study of the Individual Series.

Series 109, 6.2^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 27 and
Medial aspect, fig. 28

The cephalic arch is bent in a wide curve ventro-caudad. The tributary *A-B* of the convexity of the arch is of large size, and has, relatively, moved forward with the better differentiation of the arch from the straight segment of the precardinal. The area of the precardinal which receives the dorsal tributaries **1, 2** and **3** (in figs. 27 and 28), is somewhat expanded and dilated along its dorsal circumference, a feature which characterizes this region of the precardinal preparatory to the separation therefrom of a secondary channel by crystallization of the perivenous capillary network. As a result of this expansion of the precardinal vein, the first three dorsal tributaries of the latter (**1, 2** and **3,** figs. 27 and 28), assume a funnel-shaped form and frequently become confluent at their precardinal termination. This funnel-shaped enlargement of the terminals of dorsal tributaries **1, 2** and **3,** is well shown in fig. 27, where tributary **1** opens into the precardinal as an independent vessel, while tributaries **2** and **3** are confluent at their bases, although a small vascular island, apparently belonging to the latter, appears detached. A similar detached venous element lies between tributaries **1** and **2**. These detached vascular areas are of common occurrence in later stages, as portions of the secondary capillary plexus entering into the formation of the jugular lymph sacs. Caudal to tributary **3** the presence of a small fenestra in the precardinal vein indicates the condensation of the perivenous plexus to form the beginning of a small secondary channel. Dorsal tributary **4,** as in series 30 and 31 (figs. 22, 23 and 24) is still the largest and most prominent of the series of precardinal dorsal branches. As a whole it presents a condition preliminary to its differentiation into a medial dorsal somatic and a lateral veno-lymphatic component. In addition to its primary point of communication with the precardinal, at *X* in fig. 27, it now also opens caudad into the promontory. This condition, as

compared with the preceding series 30 and 31, has been developed as the result of the formation of the elongated fenestra *Y* (figs. 27 and 28), by means of which a dorsal para-precardinal channel has become separated from the main precardinal vein in the area between the promontory and the primary precardinal connection (*X*) of tributary **4** (fig. 27, *X*). This para-precardinal channel may have been formed as the result of the caudal extension along the precardinal of a fenestral process in the terminal of tributary **4,** similar to that present in series 30 (fig. 23).

It is not possible to state definitely what the subsequent fate of tributary **4** and its associated para-precardinal channel would have been in this specific instance, if the embryo had continued to develop. Tributary **4** would, however, be potentially capable of separating into a dorsal somatic (**4S** in fig. 11A) and a veno-lymphatic component (**4VL** in fig. 11A), the former opening into the promontory as the first of the series of promontorial somatic tributaries, and the latter entering into the formation of the caudal or posterior division of the ventral veno-lymphatic plexus, as well as contributing to the forward extension of the dorsal veno-lymphatic plexus (fig. 13).

The promontory is a capacious sac, formed largely by the dilated proximal end of the postcardinal vein and is continued ventrally into the duct of Cuvier. The dorso-medial aspect of the promontory and of the postcardinal vein receives at regular intervals the dorsal somatic tributaries **5S, 6S, 7S,** and **8S** (fig. 28, medial aspect).

The dorso-lateral aspect of the promontory (figs. 27 and 28) presents a most typical picture of the redundant venous development of this stage. It is occupied by an elongated capacious and fenestrated sac (dorsal veno-lymphatic plexus) in wide open communication with the main vein, continued caudad into the anlage common to the primitive ulnar vein and the primitive ulnar veno-lymphatic. A series of five fenestrae run along the junction of the dorsal veno-lymphatic plexus and the promontory at irregular intervals, and are continuous with those which separate the anlage common to the primitive ulnar vein and veno-lymphatic from the postcardinal.

The dorsal veno-lymphatic plexus opens into the promontory at four points, separated by fenestrae of varying width. The anlage common to the primitive ulnar vein and its associated veno-lymphatic has separated from the postcardinal to a point about midway between the dorsal somatic tributaries **7S** and **8S** (**7S** and **8S** in fig. 28), where it arches over the sixth segmental nerve to open into the dorso-lateral circumference of the promontory, and to become continuous in front with the dorsal veno-lymphatic plexus. The external jugular anlage (figs. 27 and 28) still opens into the cardinal end of the duct of Cuvier, which has not, as yet, been incorporated in the promontory.

The chief features of the left side of this embryo, as compared with the preceding earlier stages, may be enumerated as follows:

1. More marked differentiation of cephalic arch from succeeding segment.

2. Confluence and dilatation (with beginning fenestration) of terminals of dorsal precardinal tributaries **1, 2, 3.**

3. The establishment of a secondary promontorial connection for precardinal tributary **4.**

4. Clear differentiation of the jugular promontory from the straight portion of the precardinal and dilatation of the dorsal veno-lymphatic plexus. More marked separation from the postcardinal, by condensation of the capillary plexus, of the anlage of the primitive ulnar vein and veno-lymphatic.

5. Assignment of three dorsal somatic tributaries (**5S, 6S, 7S**) to dorso-medial aspect of promontory (fig. 28).

6. Differentiation of tributary **8S** as first dorsal somatic branch of the postcardinal, caudad of primitive ulnar entrance.

It is necessary to compare the lateral and medial aspects of the reconstruction in order to see the special features presented in this stage. They are shown in figs. 27 and 28.

Series 109, 6.2^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 29 and
Medial aspect, fig. 30

The reconstruction of the right side of this embryo corresponds in general with that of the left. The veno-lymphatic development and the incident plexiform formation in the area of tributary **4** shows, however, a greater degree of development.

The first three dorsal tributaries of the precardinal (**1, 2** and **3,** figs. 29 and 30) are dilated and funnel-shaped, the second and third having fused at their bases. This funnel-shaped form precedes their separation into veno-lymphatic and dorsal somatic components, the former of which will constitute the anlages of the cephalic division of the ventral veno-lymphatic plexus. As on the left side of this embryo (fig. 27), dorsal tributary **4** (**4** in figs. 29 and 30), has obtained a promontorial connection through the formation of a secondary channel (para-precardinal channel), separated from the precardinal vein by the elongated fenestra *Y* (fig. 30). In addition, a second fenestra (fig. 30, *Z*) has developed at the promontorial end of the para-precardinal channel, so that the latter is now composed in this region of two parallel vessels opening separately into the promontory. The plexus at this stage presents the condition of tributary **4** just prior to its separation into definite veno-lymphatic and dorsal somatic components, the former associated with the para-precardinal channel to form with it the caudal division of the ventral veno-lymphatic plexus.

The dorsal veno-lymphatic plexus, as on the left side of this embryo, is an extensive plexiform sac occupying the dorso-lateral aspect of the promontory (figs. 29 and 30). The sac projects forward over the precardinal vein and is separated from the promontory by a row of six fenestrae between which it communicates at seven points with the main venous system. The anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic has

not separated from the postcardinal as far forward on the right side (fig. 29) as on the left (fig. 27). In other respects its relations to the dorsal sac and the main systemic veins are the same. Figs. 28 and 30, which represent the medial aspects of the left and right sides, respectively, clearly show the relations of the dorsal veno-lymphatic plexus and the dorsal somatic tributaries (**5S, 6S, 7S** and **8S**) to the promontory and postcardinal vein. In this stage, the first of the promontorial dorsal somatic tributaries is the fifth of the entire precardino-postcardinal series (**5S**).

In a subsequent stage the fourth of the entire series (**4S**) is the first promontorial branch (compare figs. 28 and 30 with fig. 45).

In the embryo at present under consideration tributary **4,** as a whole, is still included, together with the para-precardinal channel, in a common plexus, which will subsequently differentiate into a veno-lymphatic portion (para-precardinal channel plus veno-lymphatic component of **4**) and into a somatic element. The former will constitute the caudal division of the ventral veno-lymphatic plexus. The latter, as the somatic component of tributary **4,** will enter the promontory as the first of the promontorial dorsal somatic tributaries (**4S**) in line with the rest of the series (**5S, 6S, 7S,**) and independently of the caudal division of the ventral veno-lymphatic plexus.

This ontogenetic transferal of the fourth dorsal somatic tributary to the promontory is illustrated diagrammatically in figs. 8, 9, 10, 11 and 11A, and is further explained in detail in dealing with series 138 presently to be considered. As stated above the dorsal somatic tributaries (**5S, 6S,** etc.), of the promontory do not enter into the formation of the veno-lymphatic plexus.

The embryo also exhibits well the early disposition of the veno-lymphatic plexus to shift distinctly to the *dorso-lateral* circumference of the precardinal and promontory, while the somatic tributaries are grouping themselves into a regular series entering the *dorso-medial* aspect of the promontory.

Series 2, 7^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 31 and
Reconstruction of left side,
Lateral aspect, fig. 32

This embryo, although of the same crown-rump measurement as the following series 138 (figs. 33 to 36), is less developed than the latter in so far as its veno-lymphatic elements are concerned. There is also considerable variation present upon opposite sides of the embryo, the right side having progressed to a lesser extent than the left.

On the right side the dorsal precardinal tributaries **1** and **2** (fig. 31), are expanded and plexiform at their terminals, and represent a stage preparatory to their final separation into dorsal somatic and veno-lymphatic components, as seen in series 138 (figs. 33 and 36). (Also compare with schematic diagram, fig. 10, which corresponds in this respect to the condition presented by this embryo).

Precardinal tributary **3** is not represented on the right side by any well-defined vessel. It may have become incorporated in the funnel-shaped expanded terminal of **2**, as in series 109 (fig. 29), or subsequently detached from the same, as in fig. 28, in which case it may be represented on the right side in series 2 by the detached element labelled **3VL** (fig. 31).

Precardinal tributary **4** presents essentially the same conditions as on the left side of the 6.2 mm. embryo (series 109, fig. 27). It opens cephalad into the precardinal vein at *X* and caudad into the promontory. The former connection (fig. 31, *X*) represents its original junction with the precardinal vein (compare series 30 and 31, figs. 22, 23 and 24). Its connection with the promontory has been secondarily acquired by the condensation of the precardinal plexus into a para-precardinal channel, which, separated from the main vein by the fenestra *Y* (fig. 31, *Y*), spans the interval between the promontory and the primary terminal of **4** (fig. 31, *X*), and now drains the greater part of the blood current of **4** directly caudad into the promontory.

As will be seen by comparison with the next series 138 (fig. 35), tributary 4 on the right side of the embryo now under consideration represents a stage in which the capillary plexus surrounding its terminal has not yet been clearly defined into somatic and veno-lymphatic components. A comparison of this reconstruction with the one shown in fig. 35 may, however, justify the inference that the irregular, bifurcated dorsal portion of tributary 4 (4VI, fig. 31) may represent a veno-lymphatic anlage, while the dorsal somatic component (4S in fig. 31,) in this case would separate subsequently from the veno-lymphatic component of tributary 4 and from the para-precardinal channel, and gain an independent entrance into the dorso-medial aspect of the promontory. This is one of the instances, so frequently encountered in the course of this investigation, in which it is evident that the embryo in question has been fixed during a period of transition from one well-defined stage to the succeeding condition. If we were basing our conclusions on a small number of sectioned and reconstructed embryos, it would be necessary to record the observed conditions without venturing a positive interpretation. Our material, however, has been so extensive and conclusive, and so representative of each important ontogenetic phase, that we feel justified in foretelling the significance of conditions which, in a given embryo, unmistakably point toward the stage, which, without interruption, would have been attained.

We may be pardoned if, in connection with the series under discussion, we venture to emphasize the importance of embryological investigations based on the careful analysis of a large number of embryos of approximately the same age. Our previous experience in the study of the embryonic venous variations of the cat has proved conclusively to us that it is necessary to determine normal ontogenetic conditions for each main developmental period, and that the question of embryonic departures from this norm equals in importance and significance the value which should be assigned to variations of the adult vascular system.

On the left side of this embryo (fig. 32) the ventral curvature of the cephalic arch is well established.

A-B enters as a large single vessel at the junction of the cephalic

arch and straight portion of the precardinal. The precardinal receives dorsal somatic tributaries **1, 2, 3** and **4.**

Several detached vascular islands, notably between *A-B* and tributary **2** are present. The para-precardinal channel has been separated from the main precardinal vein independently of tributary **4,** or, if at one time connected with it, this connection has been lost. In other words, the embryo under discussion is one of the instances illustrating the type of development described in the analysis (cf. p. 215, fig. 19), in which the caudal division of the ventral veno-lymphatic plexus is apparently derived entirely from the para-precardinal channel, without involving tributary **4.** In the present instance, the elongated fenestra *Y* in fig. 31, between the precardinal and promontorial terminals of the para-precardinal channel, is replaced on the left side of the embryo by two rectangular foramina (fig. 32, *Y'* and *Z*); consequently the para-precardinal channel here drains through the resulting inter-fenestral pathways, by three separate and distinct openings into the main channel.

The fenestral character of the terminal of tributary **4** (**4,** fig. 32), indicates that a secondary channel is being formed along the precardinal vein, independently of the para-precardinal channel, by means of which tributary **4** will subsequently drain into the promontory.

The dorsal veno-lymphatic plexus is more advanced in development on the left side than on the right. It is continuous cephalad with the caudal division of the ventral veno-lymphatic plexus, and caudad with the anlage common to the primitive ulnar vein and the primitive ulnar veno-lymphatic. This anlage has differentiated completely from the postcardinal vein, caudal to the termination in the latter of dorsal somatic tributary **7** (fig. 32, **7S**). The anlage of the permanent external jugular system is represented on the left side by a single trunk (fig. 32), corresponding to the double vessel found on the right side (fig. 31) in the same situation.

Compared with the preceding series (109, 6.2mm., figs. 27 and 29), the right side of this embryo (fig. 31) shows retarded development of the dorsal veno-lymphatic plexus. A single channel, closely following the dorso-lateral circumference of the promon-

tory, and only differentiated from the same by a linear series of three fenestræ, represents the extensive plexiform area which in series 109 (fig. 27) occupies the dorso-lateral circumference of the promontory. The same relative reduction of the dorsal veno-lymphatic plexus is seen on the left side of this embryo (fig. 32) when compared with the same side of the 6. 2 embryo (series 109, fig. 27.)

Series 138, 7^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 33 and
Medial aspect, fig. 34

This embryo offers a remarkably clear picture of veno-lymphatic development and illustrates well the relation of the veno-lymphatic plexuses to the system of the primitive dorsal somatic tributaries.

The plexiform and dilated terminals of precardinal tributariēs **1**, **2** and **3** (as seen in figs. 27, 29 and 31), and the terminal of dorsal precardinal tributary **4** and its associated para-precardinal channel, reveal here very strikingly their separation into dorsal somatic and veno-lymphatic components. Another feature of fundamental importance to the interpretation of succeeding stages is the tendency displayed by these veno-lymphatic derivatives to shift their connection with the precardinal from the dorsal to the dorso-lateral circumference of the main channel.

After these veno-lymphatic derivatives have assumed their typical dorso-lateral position with respect to the main systemic venous channel, they are then in line to fuse with one another and with the dorsal veno-lymphatic plexus which, from the beginning, projects cephalad from the dorso-lateral circumference of the promontory.

This embryo likewise throws considerable light on the genesis of the variations presented by the terminal of dorsal somatic tributary **4** of the early series, in reference to the double character assumed by the same in both the somatic and the veno-lymphatic direction. For these reasons the embryo requires special and detailed consideration.

Tributary 1 (fig. 33, lateral aspect) enters the cephalic end of the straight precardinal segment by two branches, inclosing a wide fenestral space. The caudal of these two branches (fig. 33, 1VL) opens into the dorso-lateral circumference of the precardinal, a circumstance which, in addition to its plexiform and dilated character, designates it as a veno-lymphatic structure. Here the crystallization of the plexiform reticulum into definite somatic and veno-lymphatic lines is still evident within the basal area of the tributary.

Tributary 2 is represented by a veno-lymphatic component (2VL) which opens into the dorso-lateral, and by a dorsal somatic component (2S, fig. 34), which opens into the dorso-medial circumference of the precardinal.

The original anastomosis between these components of 2 has in this instance been lost, and they now open into the precardinal as separate and independent tributaries.

This interpretation is supported by the disposition of the succeeding branches in this embryo.

Dorsal somatic tributary 3 has separated into a lateral veno-lymphatic (3VL) and a medial dorsal somatic component (3S). The veno-lymphatic component (3VL) consists of a large expanded double branch (3VL) and of a smaller caudal division (3VL′). Both of these veno-lymphatic elements open into the dorso-lateral circumference of the precardinal.

The dorsal somatic component of tributary 3 (3S) is extremely small, and its relation to the veno-lymphatic element of this tributary clearly indicates its original connection with the same.

The veno-lymphatic components of the first three dorsal precardinal tributaries constitute the anlage of the Cephalic or Anterior Division of the Ventral Veno-Lymphatic Plexus. (Cf. figs. 10, 11, 12 and 13).

The caudal or posterior division of the ventral veno-lymphatic plexus is already fully established. It is formed by the para-precardinal channel and by the veno-lymphatic components of 4 (4VL in figs. 33 and 34); while the dorsal somatic element of 4 (4S in figs. 33 and 34) now opens into the dorso-medial aspect of the promontory.

The veno-lymphatic element of 4 represents the fenestrated

part of the terminal of the early branch 4 in a more advanced state of development. It has retained a cephalic connection with the precardinal (*X*, fig. 33), but the para-precardinal channel has broken away at the usual distal tap (at *B*) into the angle between the precardinal and promontory, apparently by secondary fusion at this point with the dorsal veno-lymphatic plexus.

This embryo may possibly offer the ontogenetic explanation of the adult cases in which the lymphatic system, through the jugular lymph sac, communicates with the venous system only at the distal of the two customary taps, viz., at the jugulo-subclavian angle. By early union of the elements of the ventral veno-lymphatic plexus with the dorsal plexus, the terminal of the latter, at the jugulo-subclavian angle, may lead secondarily to the formation of the only definite adult lymphatico-venous portal. As previously stated (p. 192) the results of this investigation oblige us to assume that the early embryonic connections of the veno-lymphatic plexuses with the permanent veins are given up and that the adult lymphatico-venous taps are formed secondarily.

Compared with series 109 (figs. 27 and 30) this side of the embryo clearly shows the double character now assumed by precardinal tributary 4 and the associated para-precardinal channel (figs. 33 and 34), and the lines along which the veno-lymphatic and dorsal somatic components separated. On the left side of this embryo this separation is complete, except at one point (figs. 33 and 34, *A*) where the original connection between the two still persists.

The veno-lymphatic component of 4 (4VL in fig. 33) and its associated para-precardinal channel is expanded and dilated. It has, as in series 109 (figs. 27 and 30), retained its original point of connection with the precardinal vein (*X* in fig. 33). The promontorial opening of the para-precardinal channel at the promontorio-precardinal angle has been much reduced, owing perhaps to the dorsal extension of the fenestra *Y*. (fig. 33), resembling somewhat the condition found in series 109 (fig. 30). The small curved tributary *B* in fig. 33 at the promontorio-precardinal angle, probably represents a portion of the para-precardinal channel separated from the main vessel by the extension of fenestra *Y*. The veno-

lymphatic complex of 4 and of the para-precardinal channel has become confluent with the dorsal veno-lymphatic plexus.

An elongated diverticular process near the promontory (*A P*, fig. 33) opens on the left side into the para-precardinal channel. The same element is present on the right side (*AP*, fig. 35) but, in this case, does not terminate in the para-precardinal channel, but in the dorsal somatic component of precardinal tributary 4 (4S) which is still but incompletely differentiated from the para-precardinal channel.

The dorsal somatic component of 4 (4S, fig. 34) is composed of two parallel channels, united anteriorly by a short transverse branch, which open into the dorso-medial circumference of the promontory by a common trunk. The ventral and longer of these two channels follows closely the dorso-medial circumference of the precardinal vein and terminates cephalad in a blind extremity, near which it communicates (figs. 33 and 34, at *A*) with a veno-lymphatic component (4VL) of tributary 4. About midway in its course the ventral channel also communicates with the precardinal vein (fig. 34, *X'*), thus retaining its primary connection with the main vein as in figs. 22, 23 and 24, as does also the veno-lymphatic derivative of tributary 4 (fig. 33, *X*). The dorsal smaller subdivision of the somatic element of tributary 4 terminates cephalad in a blind end.

The dorsal division of the veno-lymphatic plexus is an extensive multi-fenestrated product of the dorso-lateral portion of the promontory, the plexiform network extending caudad along the line of the future separation of the primitive ulnar from the main postcardinal vein.

The promontory in series 138 (figs. 33 to 36) is a well-defined area. The external jugular anlage opens into the promontory near the latter's point of confluence with the duct of Cuvier.

Series 138, 7^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 35 and
Medial aspect, fig. 36

The right side of the embryo presents the same clear differentiation into dorso-lateral veno-lymphatic and dorso-medial

somatic precardinal elements, but with a number of communications still persisting between the corresponding elements of the two sets.

Precardinal dorsal tributary 1 is fenestrated at its junction with the main vein, so as to open into the same by two interfenestral channels (1VL, fig. 35). It occupies the dorso-lateral aspect of the vein and may be fairly regarded as the most anterior veno-lymphatic element. There is no corresponding dorso-medial or somatic branch.

The dorsal precardinal tributary 2 is represented by a dorso-lateral, dilated veno-lymphatic (2VL), which opens dorso-laterally into the precardinal vein (fig. 35), by two small detached veno-lymphatics (2VL′, figs. 35 and 36), and by a small dorsal somatic branch (2S, fig. 36) which opens dorso-medially into the precardinal.

The precardinal dorsal tributary 3 is still in the plexiform condition, and although it has not as yet divided into separate and independent veno-lymphatic (3VL) and dorsal somatic (3S) components, as in the case of tributary 2, these components can be clearly differentiated from each other on account of the relations which they bear to the dorso-lateral (3VL) and dorso-medial (3S) circumference of the precardinal vein (figs. 35 and 36).

The two detached vascular elements which lie dorsal to the area of tributary 2, on account of their dorso-lateral position, are veno-lymphatics and have probably seen separated from the veno-lymphatic component of tributary 3.

All of these veno-lymphatic components of tributaries 1, 2 and 3 constitute the anlage of the cephalic division of the ventral veno-lymphatic plexus (cf. figs. 11 and 12).

In the area of the complex formed by the precardinal tributary 4 and its associated para-precardinal channel (caudal division of the ventral veno-lymphatic plexus) we encounter an excellent example of the incomplete separation between the veno-lymphatic and dorsal somatic components into which the early complex has subdivided, as the two sets of components are still connected with each other by means of a number of transverse vessels.

The dorso-lateral veno-lymphatic component of tributary 4 (4VL, fig. 35) appears as an extensive sac, which ends anteriorily in a

blind diverticular extremity. When compared with the reconstruction of the left side (fig. 33) it appears possible that this diverticular extremity may have been derived from tributary **3**. Such a condition might easily be brought about as the result of a fusion between the veno-lymphatic components of tributaries **3** and **4**. Measurements of the two reconstructions seem to bear out this view. (See also **3VL**, in fig. 47).

The dorso-laterally situated veno-lymphatic complex of tributary **4** (**4VL**) and its associated para-precardinal channel (para-precardinal channel, **VL**) communicate at three points on the right side of this embryo with the main precardinal vein (at *X*, *X'* and *X''*, figs. 35 and 36). The most anterior point of communication at *X''* (fig. 35) is not present on the left side of the embryo (fig. 33) unless, as intimated above, it may be represented by the precardino-veno-lymphatic confluence of tributary **3**. The communications with the precardinal at *X* and *X'*, however, are represented on both sides of the embryo, as is evidenced by comparative measurements of the two sides. (Compare *X* and *X'* in figs. 33, 34 and 35).

On the right side of the embryo the communication with the precardinal at *X* occurs dorso-medially and in common with a secondary somatic component of tributary **4** (**4S'**, fig. 36), while on the left side of the embryo the communication with the precardinal at *X* occurs dorso-laterally and independently of a somatic branch (*X*, fig. 33).

The same reverse conditions prevail regarding the point of communication with the precardinal at *X'*, since it occurs dorso-laterally on the right side (*X'*, fig. 35) and dorso-medially on the left (*X'*, fig. 34).

The communication at *X* on both sides of the embryo probably represents the persistence of the primary union of tributary **4** with the precardinal (see series 109, *X*, fig. 28), while the communication at *X'* probably represents an instance in which the para-precardinal channel has not been completely separated along its entire extent from the precardinal vein.

Caudally, the veno-lymphatic complex of tributary **4** and its associated para-precardinal channel enlarges and presents a multi-

ple plexiform formation, in line with the fenestrae along the promontorial and primitive ulnar areas, and is directly continuous with the dorsal veno-lymphatic plexus (fig. 35). The position occupied by the fenestræ in the complex suggest that the latter may subsequently be separated into a number of parallel channels (see fig. 45).

Medially, the veno-lymphatic complex of tributary 4 and the para-precardinal channel communicates by means of six transverse branches (**a, b, c, d, e** and **f**, figs. 35 and 36) with a large dorso-medial somatic trunk (**4S**) which, as the chief representative of the dorsal somatic element of precardinal tributary 4, now enters the promontory at the promontorio-precardinal angle and forms the first of a series of segmentally arranged promontorial dorsal somatic tributaries (**4S, 5S,** and **6S,** figs. 36). The promontorial dorsal somatic tributary **7S** is not shown in the figure.

The presence of the above mentioned transverse communications at **a, b, c, d, e,** and **f** (figs. 35 and 36) represents a stage of development in which the separation of the early complex of precardinal tributary **4** and its associated para-precardinal channel into its veno-lymphatic and dorsal somatic components is still incomplete, and more so than on the left side of the embryo, where only one such connection exists (*A*, figs. 33 and 34).

Fig. 37 is a scheme of the right side of series 138 (fig. 35) viewed from its dorsal aspect, in which the dorso-lateral (veno-lymphatic) and dorso-medial (somatic) components of tributaries **1, 2, 3** and **4** have been slightly divaricated in order to expose the dorsal surface of the precardinal and promontory and to emphasize, thereby, the double character of the precardinal tributaries, as well as the relation subsequently assumed by their veno-lymphatic and somatic components with respect to the main venous channels.

The legends in fig. 37 correspond to those in figs. 35 and 36. The following description of the diagram (fig. 37) will make clear the complicated conditions observed in series 138.

In the area of primitive dorsal tributary **4** we encounter, in its fullest degree, the development of the dorso-lateral veno-lymphatic component, of the dorso-medial somatic branch, and of the transverse communications between the two. The dorso-lateral

veno-lymphatic element, (**4VL,** fig. 37) appears as an extensive and fenestrated sac. It ends anteriorly in a blind diverticular extremity. Two channels of communication through X'' and X' lead into the main precardinal channel, between which a transverse branch connects a medial somatic tributary (**4S'**) with the veno-lymphatic component of tributary **4** (**4LV**), Proceeding caudad, the veno-lymphatic sac of **4** enlarges and presents multiple fenestral formation in line with the fenestræ of the promontorial and primitive ulnar areas, and unites with the redundant veno-lymphatic dilatation of this region (dorsal veno-lymphatic plexus). Medially it connects by six transverse branches (**a, b, c, d, e, f**) with a well developed dorso-medial trunk (**4S**) which, as the main representative of the somatic element of **4**, enters the dorso-medial angle of the promontory in line with the succeeding dorsal somatic promontorial branches **5S** and **6S**, etc.

It will be noted that in the general plan of organization the two sides of this embryo agree closely and present a consistent picture, characterized by three main features.

1. Distinct development of a dorso-lateral series of dilated veno-lymphatic elements derived from the precardinal tributaries **1, 2** and **3** and from precardinal tributary **4** and its associated para-precardinal channel. Of these the derivatives of tributaries **1, 2** and **3** represent the anlage of the cephalic division of the ventral veno-lymphatic plexus, while the element derived from the tributary **4** and the para-precardinal channel forms the anlage of the caudal division of the ventral veno-lymphatic plexus. In early stages the latter is often continuous with the dorsal veno-lymphatic plexus, which is derived from the dilated, plexiform dorso-lateral aspect of the promontory and the primitive ulnar segment of the postcardinal.

2. Coincident with this development is the appearance of a series of dorso-medial branches which represent the somatic elements of the primitive precardinal tributaries. These are very variable in their character, as may be seen by comparing the opposite sides of the embryo (figs. 34 and 36).

3. The most remarkable feature is the persistence between the dorso-lateral veno-lymphatic and dorso-medial somatic elements of a series of transverse communications.

The embryo illustrates exceedingly well the fact that, in certain stages, or in certain individuals, while the early dorsal tributaries **1, 2** and **3** are largely taken up in the veno-lymphatic organization and shifted to the dorso-lateral circumference of the precardinal, portions of the early elements may remain as small and temporary channels in line with the succeeding dorsal somatic series, and function to a limited extent as dorsal somatic tributaries of the precardinal.

Moreover, this embryo clearly establishes the compound character of the element **4**, and its associated para-precardinal channel, and accounts for the many variations encountered in its connections with the main channels and its relation to the veno-lymphatic sac. In fact this entire question can best be discussed on the hand of the conditions shown by the reconstruction just described, compared with other typical series.

Series 13, 7.25^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 38 and
Reconstruction of left side,
Lateral aspect, fig. 39

This embryo is especially interesting for the reason that it illustrates very clearly the range of variation, as regards veno-lymphatic development, which may be presented on opposite sides of the same embryo.

On the right side of the embryo (fig. 38) the straight segment of the precardinal receives at regular intervals three well-defined dorsal tributaries (**1, 2** and **3** in fig. 38). In front of its confluence with tributary **2** the precardinal is much dilated.

Precardinal tributary **4** of the right side is represented by two veno-lymphatic structures (**4VL** and **4VL′**, fig. 38). One is a triangular-shaped element(**4VL′**) which rests upon the dorsal surface of the precardinal at the promontorio-precardinal angle and lies in line with the precardinal tributaries **1, 2** and **3**. As far as we are able to determine this structure does not communicate with the venous system and may, in this case, constitute the anlage of the dilated, rounded appendage of the ventral divi-

sion of the veno-lymphatic plexus, which occupies a similar position and is so often met with in later stages (see series 77, fig. 51). The other veno-lymphatic component (**4VL**) of precardinal tributary **4** is the larger of the two. It occupies a dorso-lateral position, has given up its connection with the precardinal and opens into the promontory by a right-angled line which is directly continuous with that of the dorsal veno- lymphatic plexus.

This embryo shows a condition which can be derived from that obtaining in series 30 and 31 (figs. 23 and 24) by development of a para-precardinal channel involving the fenestrated terminal of tributary **4** and the subsequent detachment of the complex thus formed from the precardinal and promontory back to the point where if becomes continuous with the dorsal veno-lymphatic plexus.

The right-angled limb of this veno-lymphatic structure shows clearly the course along which the process of fenestration proceeded. *We have here an instance, in all probability, in which the precardinal tributary* **4** *has been transformed in its entirety into veno-lymphatic components.* If such is actually the case, dorsal somatic tributary **5S,** as in the present case (fig. 38), will form the first of the promontorial tributaries.

Veno-lymphatic formation along the dorso-lateral circumference of the promontory and postcardinal vein is well advanced on the right side of this embryo except for an area which intervenes between the dorsal veno-lymphatic plexus and the anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic. This anlage opens into the main channel at three points in the neighborhood of the termination of the promontorial dorsal somatic tributary **7S** (fig. 38). Caudad of these connections the primitive ulnar vein has completely separated from the postcardinal.

The promontory is not as yet a capacious sac and is formed on the right as well as upon the left side (fig. 39) by the enlarged Cuvierian end of the postcardinal. The anlage of the external jugular vein opens on each side into the proximal end of the duct of Cuvier, a circumstance which denotes the still incomplete development of the promontory.

Series 13, 7.25^{mm} Embryo
Reconstruction of left side.
Lateral aspect, fig. 39

The two salient features of this embryo which distinguish the left side (fig. 39) from the right (fig. 38) are that on the left side the dorsal precardinal tributaries, with the exception of tributary **1**, are either rudimentary or are practically wanting as such, and that the promontorial end of the straight segment of the precardinal consists of two parallel channels, subequal in caliber, which are separated from each other by an elongated fenestra (Y, fig. 39).

The dorsal precardinal tributary **1** (fig. 39) is a vessel of fairly large size. It opens dorso-medially into the precardinal and as yet shows no sign of fenestration.

Precardinal tributary **2** is represented by a veno-lymphatic element, which opens dorso-laterally into the precardinal (**2VL,** fig. 39) and probably by the detached element which lies contiguous to the latter.

Precardinal tributaries **3** and **4**, as distinct branches of the precardinal, are wanting, as no trace of any tributaries opening into the precardinal could be found in the territory usually occupied by them. In their place, however, and occupying a dorso-lateral position with respect to the para-precardinal channel and in line with the dorsal veno-lymphatic plexus, are situated a number of independent veno-lymphatics (**3, 4VL,** fig. 39) which probably represent these tributaries, and which in further development would probably contribute to the formation of the continuous veno-lymphatic arch of later stages, which lies dorso-lateral to the straight segment of the precardinal.

The separation of the promontorial end of the straight segment of the precardinal into two parallel channels is a feature of development which has already been described in connection with series 109 (fig. 28) and series 2 (fig. 31). Usually, however, the more dorsal of the two channels, which we have termed the para-precardinal channel, is the smaller of the two and serves, at a certain period of development, as the main pathway through which the precardinal tributary **4** drains into the promontory. In the

present instance, having lost its connection with the precardinal tributary 4, it functions in a manner similar to that of the more ventrally situated main precardinal channel and represents an advanced stage in the separation from the latter of what constitutes the anlage of the caudal division of the ventral veno-lymphatic plexus.

The veno-lymphatic development along the dorso-lateral circumference of the promontory and postcardinal vein has progressed to a slightly greater extent than on the right side. The broken line along the promontory (fig. 39) shows the extent to which the veno-lymphatic ridge projects from the promontory. The anterior end of the dorsal veno-lymphatic plexus extends forward over the para-precardinal channel, where it ends blindly and is continuous caudad with the anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic. This vessel opens into the postcardinal by means of a single channel, and caudad of this point, as on the right side, it has completely separated from the postcardinal.

We have here an instance, as on the right side of this embryo, in which *the first promontorial dorsal somatic tributary* (*5* **S**, *fig. 39*) *is represented by the fifth of the series, and in which, in all probability, precardinal tributary* **4** *has been transformed in its entirety into veno-lymphatic components which will aid in the forward extension of the dorsal veno-lymphatic plexus. Also, on the left side, fig. 39, a case in which the caudal division of the ventral veno-lymphatic plexus is derived solely from the para-precardinal channel.* Compare figs. 39 and 40.

SUBSEQUENT EARLY STAGES ILLUSTRATING VENO-LYMPHATIC DEVELOPMENT

Series 102, 8.5^{mm} Embryo
Series 106, 9^{mm} Embryo
Series 19, 9^{mm} Embryo

This group of embryos presents the following features of veno-lymphatic development in advance of those described in connection with the preceding group:

1. As the result of its caudal extension, the promontory has become a capacious sac, involving in its caudal growth the proximal end of the duct of Cuvier and of the postcardinal vein (see fig. 8). The permanent external jugular anlage now opens into the promontory.

2. Precardinal dorsal tributaries **1, 2, 3** and **4** are completely differentiated into definite veno-lymphatic and dorsal somatic components.

3. The veno-lymphatic components of precardinal tributaries **1, 2** and **3** have become much dilated, and secondary plexus formation has now invaded the anterior portion of the straight segment of the precardinal.

4. In addition to the two veno-lymphatic plexuses met with in the preceding group (dorsal plexus and caudal division of ventral plexus) the cephalic division of the ventral plexus makes its first definite appearance in the group under consideration.

Embryo 102, in spite of its smaller measurement (8.5 mm.), possibly due to greater curvature, offers more advanced developmental conditions than the remaining members of the group. It is therefore desirable to consider the last two series first.

Series 196, 9^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 40

This embryo illustrates very well a condition frequently observed in the early stages, of comparatively advanced development of the veno-lymphatic area in one section, with retarded development in another.

Tributary *A-B* forms a prominent trunk with smaller accessory branches (**b, b′**) entering the convexity of the cephalic arch caudal to its termination.

Only the veno-lymphatic components of the precardinal dorsal tributaries **1, 2** and **3,** are shown in the figure. Two prominent dorsal somatic tributaries open into the dorso-medial circumference of the precardinal in the area occupied by tributaries **1** and **2.** These lie in a line with the series of promontorial and postcardinal dorsal somatic vessels and have been derived from the

plexus of the dorsal precardinal tributaries of earlier stages as the result of the condensation into dorsal somatic and veno-lymphatic components (see description of series 138, and figs. 33 and 35).

The veno-lymphatic component of tributary 1 (1VL, fig. 40) is dilated dorsally into an elongated sac which opens into the dorso-lateral circumference of the precardinal by a slender channel. From its general appearance and relations to the area of the precardinal occupied by precardinal tributary 2, it is possible that this dilated sac may be a compound structure formed through a fusion of veno-lymphatics derived from tributaries 1 and 2. After fusing with the more caudally situated veno-lymphatics, however, it will form the most anterior portion of the continous dorsal veno-lymphatic sac of later stages and its primary connection with the precardinal vein may serve as the channel of exit into the systemic veins of the blood contained in the veno-lymphatic sac (see figs. 12, 13, 14 and 15).

The area of the precardinal occupied by tributary 2 (2 VL) is redundant and fenestrated, a condition which foreshadows the formation of an anterior para-precardinal channel which may involve in its separation from the precardinal the bases of tributaries 1, 2 and 3, At a later stage this channel frequently fuses along the lateral surface of the precardinal with an anterior prolongation of the caudal division of the ventral veno-lymphatic plexus (see figs. 12, 13, 14 and 15).

The veno-lymphatic component of precardinal tributary 3 (3VL) is, relatively, a large and somewhat dilated, single vessel which opens dorso-laterally into the precardinal, caudal to the territory occupied by tributary 2 (2VL).

Precardinal tributary 4 presents an interesting condition in this embryo and one which, in some respects, resembles that found on the right side of the 7.25 mm. embryo (fig. 39), since it has severed its connection with the para-precardinal channel and the precardinal vein. The primary point of connection which formerly existed between tributary 4 and the precardinal vein (*X*, fig. 40) is indicated in the figure by broken lines.

In the area of precardinal tributary 4 and its associated para-precardinal channel the process of fenestration is completed. A

dorsal somatic branch (**4S**) has been separated from tributary **4** and the para-precardinal channel, which now opens into the dorso-medial surface of the promontory, where it forms the first of a series of promontorial somatic tributaries (**4S**, fig. 40). The veno-lymphatic component of tributary **4** (**4VL**, fig. 40) which has severed its connections with the precardinal and the para-precardinal channel, now occupies a dorso-lateral position with respect to the precardinal, and lies in line with the dorsal veno-lymphatic plexus and the veno-lymphatics derived from tributaries **1, 2** and **3,** with which it will subsequently fuse to form the dorsal veno-lymphatic arch in fig. 14. The para-precardinal channel still communicates with the precardinal and the promontory, being separated from the former by the fenestra *Y*. *The para-precardinal channel on the left side of this embryo constitutes the sole anlage of the caudal division of the ventral veno-lymphatic plexus,* precardinal tributary **4** *per se* not contributing to its formation.

As the result of a caudal extension which involved the proximal ends of the postcardinal and the duct of Cuvier, the promontory has increased in size in an antero-posterior direction. In consequence of this caudal extension, the duct of Cuvier joins the postcardinal further caudad than in the earlier stages and no longer receives the anlage of the permanent external jugular vein, which now opens into the ventro-lateral circumference of the promontory. The promontory has not yet assumed the rounded and sac-like appearance in this embryo which is so characteristic of this area in certain other members of this group.

The anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic has completely separated from the postcardinal. After arching over the sixth spinal nerve (*SP.N.VI*, fig. 40) it opens into the dorso-lateral circumference of the promontory, contiguous to the latter's point of confluence with the sixth dorsal somatic tributary (**6S**). Anterior to its union with the promontory it becomes continuous with the dorsal veno-lymphatic plexus.

The most marked advance is shown in this embryo in the dorso-lateral circumference of the promontory and of the arch of the primitive ulnar vein. This segment of the primitive venous

pathway has developed a large dilated veno-lymphatic sac representing the dorsal veno-lymphatic plexus. It forms an irregularly quadrilateral dilated and diverticular appendage, projecting from the dorso-lateral aspect of the promontory and primitive ulnar arch. The persistent pathways between the fenestral spaces furnish four channels of communication between this veno-lymphatic sac and the permanent vein. Only three of these channels are shown in the figure. This represents an advance over the conditions shown in the earlier stages in this region. The fenestræ have become elongated, thus drawing out the persistent patent intervals between them into definite and well-marked channels of communication. At a later stage of development, and in connection with the formation of the primitive ulnar veno-lymphatic, the dorsal veno-lymphatic plexus, through confluence of its fenestræ, becomes separated from the primitive ulnar vein, but retains a single point of communication with the promontory near the latter's junction with the primitive ulnar vein (see Tap *C*, figs. 40 and 44).

As previously stated, this embryo offers a marked example of the fact that veno-lymphatic development does not proceed uniformly in all parts in the primary venous area involved, and that in individual portions of this area one segment may be developmentally much in advance of the rest, in a given stage. Thus in the present instance the dorsal veno-lymphatic plexus is considerably further developed than the ventral division (para-precardinal channel), while in other examples the reverse may obtain (series 2, fig. 32). The reconstruction of this embryo also furnishes a marked instance of the multiple openings, characteristic of the intermediate stages, between the anlages of the future definite veno-lymphatic plexus and the permanent veins. With the more complete separation of the veno-lymphatic sac from the main venous channels the intervals between the individual fenestral spaces elongate and become drawn out into narrower and longer branches of communication between the two vascular areas. Thus, in the present instance, we find, caudal to the junction of tributary *A–B* with the cephalic arch, ten distinct openings leading from the anlages of the veno-lymphatic plexuses into the dorsal circumference of the precardinal vein and promontory (fig. 40).

Subsequently, as stated in the preliminary analysis of veno-lymphatic development, and as will be shown in the course of the detailed consideration of the more advanced stages, these multiple early connections undergo great numerical reduction.

This embryo, as well as series 13 (fig. 39), is also of great importance in illustrating a feature of veno-lymphatic development which, as yet, has not been carefully considered and one which is even more marked in certain of the succeeding stages. We refer to the presence of the detached and isolated veno-lymphatic sacs, or spaces which appear as outlying islands to the main divisions of the veno-lymphatic plexus and in the area in which the jugular lymph sacs are subsequently found. These detached sacs may or may not contain blood corpuscles and, in the latter case, they present the appearance of typical isolated lymphatic spaces.

The gradual separation and detachment of the main veno-lymphatic plexus from the systemic veins constitutes a uniform and typical phase in the ontogeny of the jugular lymph sacs. It, therefore, appears to us that the detachment of these small veno-lymphatic elements, in advance of the main veno-lymphatic system, may be explained on the ground that their detachment merely anticipates the main sac in attaining this stage.

As to the subsequent fate of these detached spaces there can be no doubt. They enlarge, fuse with one another and with the main veno-lymphatic plexuses in forming the anlage of the jugular lymph sac.

Series 19, 9^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 41 and
Reconstruction of left side,
Lateral aspect, fig. 42

This embryo is markedly curved and shows a distinct demarcation into cephalic arch, straight precardinal segment and promontory. It affords an instance of the developmental type described in the analysis (p. 211) and illustrated by fig. 9, in which the cephalic division of the ventral veno-lymphatic plexus is formed primarily from the peri-precardinal capillary plexus, involving

secondarily the terminals of dorsal precardinal tributaries 1, 2 and 3, which are taken up in the veno-lymphatic organization. The two sides of this embryo present two distinct stages in the condensation of the peri-precardinal plexus and the subsequent separation of the resulting anlage of the cephalic division of the ventral plexus from the main channel.

Reconstruction of Right Side, Lateral Aspect, Fig. 41.

On the right side, the area receiving the terminals of dorsal tributaries 1, 2 and 3 in fig. 41, is plexiform and redundant. A portion of this plexus is condensed into a well-defined veno-lymphatic channel partially separated from the main vein by a fenestra through which the arrow passes in the figure. This channel, the principal anlage of the cephalic division of the ventral veno-lymphatic plexus, is a much more prominent structure than its appearance would denote in the figure.

It is also significant in this embryo that dorsal somatic tributaries 1, 2 and 3 are practically not involved in the development of the cephalic division of the ventral veno-lymphatic plexus, except in so far as their expanded terminals are included in the redundant precardinal area which is responsible for the formation of this portion of the plexus.

The complex formed by the precardinal tributary 4 and its associated para-precardinal channel has separated into its dorsal somatic (4S) and veno-lymphatic (4VL) components.

The dorsal somatic component of tributary 4 (**4S**, fig. 41) opens into the dorso-medial circumference of the promontory and now forms the first of the promontorial series of somatic tributaries (**4S**, **5S** and **6S**, fig. 41).

The veno-lymphatic derivative of the primary dorsal tributary 4 (**4VL**, fig. 41) consists of an expanded, dilated and fenestrated sac which communicates with the precardinal at its primary point of connection with the latter (*X* in fig, 41; see also figs. 22 and 23) and which also drains into the promontory through a para-precardinal channel that has developed from the peri-precardinal plexus in front of and contiguous to the promontory. Strictly

speaking, this para-precardinal channel, in this instance, as well as in all others, in which it serves as a portal through which tributary 4 drains into the promontory, must be regarded as constituting a portion of tributary 4. In addition to its points of communication with the precardinal and promontory, just mentioned, the veno-lymphatic component of tributary 4 (**4VL,** fig. 41) also communicates with its companion dorsal somatic component (**4S,** fig. 41) near the point where the latter opens into the promontory, a circumstance which betrays the common origin of these two components from the primary dorsal plexus of tributary 4 of earlier stages (see figs. *11A*, 35 and 37). The small diverticular-shaped process which projects ventrad from the para-precardinal channel (*AP*, fig. 41) corresponds to a similarly situated process on the left side of series 138 (fig. 33).

In front of the primary point of connection of tributary 4 with the precardinal (*X* in fig. 41) the latter receives a small inverted L-shaped tributary (*L* in fig. 41) which opens into its dorso-lateral circumference. This small vessel is probably related to the plexus of the primary terminal of tributary 4 (*X*) from which it has been separated in the condensation of the channels. Its position with respect to the main channel establishes its veno-lymphatic character and indicates that it will enter into the formation of the ventral division of the veno-lymphatic plexus as in series 102, fig. 44.

The dorsal veno-lymphatic plexus was only partially reconstructed on the right side of this embryo and, therefore, will not be considered.

Reconstruction of Left Side, Lateral Aspect, Fig. 42.

A comparison of the right and left sides of this embryo affords an interesting illustration of the unequal chronological development of the three main divisions of the veno-lymphatic plexus which may occur upon opposite sides of the same embryo. On the left side (fig. 42) the cephalic division is much advanced in development and the caudal division of the ventral plexus extremely rudimentary, while on the right side (fig. 41) the reverse conditions obtain.

The anlage of the external jugular vein is well developed and opens into the promontory.

The anterior part of the straight precardinal segment, in the area corresponding to the dorsal tributaries 1, 2 and 3, carries on its dorso-lateral aspect a large diverticular sac, which extends caudad in the direction of the promontory. This sac, produced by condensation of the peri-precardinal capillary network, forms the anterior or cephalic division of the ventral veno-lymphatic plexus (*cf.* fig. 9). On the right side of this embryo (fig. 41) this structure is in the process of differentiating from the main vein. In the later stages it will fuse with the dorsal plexus derived from the promontory, and with the caudal division of the ventral plexus, and will then form the anterior portion of the continuous veno-lymphatic sac (fig. 42, area of 1, 2 and 3). At its anterior end it is still in wide-open communication with the precardinal and it terminates caudally in a blind and deeply bifurcated extremity which overlaps the dorso-lateral aspect of the straight precardinal. When the cephalic division of the ventral plexus is formed in this manner the precardinal tributaries 1, 2 and 3 appear to be taken up as a whole in the veno-lymphatic organization and do not divide into veno-lymphatic and somatic components as far as we can determine.

The left side of this embryo apparently represents an instance in which *primary dorsal tributary* 4 *of the precardinal has been transformed in its entirety into a dorsal somatic promontorial branch* (4S) and has not contributed a component to the ventral veno-lymphatic plexus.

It may be possible that the more dorsal of the two processes (4VL′) which, form the bifurcated extremity of the anterior veno-lymphatic sac has been derived from tributary 4 and has been secondarily joined to the sac. If such is the case, we are not in a position to prove it, and must, therefore, abide by our original contention that tributary 4 *per se*, in this particular instance, does not furnish a veno-lymphatic component.

The dorsal somatic tributary 4 (4S, fig. 42) is formed by two confluent branches which enter the dorso-medial aspect of the promontory at the promontorio-precardinal angle. The vessel is expanded and fenestrated near its promontorial connection and lies in line with a series of promontorial and postcardinal dorsal somatic tributaries (5S, 6S, 7S and 8S, fig. 42), of which it forms the first or most anterior member of the series.

The para-precardinal channel, so prominently developed on the right side in connection with the veno-lymphatic component of tributary 4 (fig. 41), is represented on the left side by a small diverticular process of the promontory which is connected with the dorso-lateral circumference of the latter (para-precardinal channel, fig. 42). An examination of the actual reconstruction of the left side shows more clearly than is indicated in the figure that this short stump-like para-precardinal element was probably continuous at one time with the more ventrally situated of the two processes which form the posterior extremity of the cephalic division of the veno-lymphatic plexus or sac, and that it has separated from the latter, during the course of development. Such as it is, however, it represents in its entirety, on the left side of this embryo, all that is present of the caudal division of the ventral veno-lymphatic plexus, which is well developed on the right side (fig. 41), as well as in certain embryos of the earlier stages (fig. 32). (Cf. also fig. 21).

The dorsal veno-lymphatic plexus of the left side (fig. 42) is extensively developed. It has given up its connection with the anterior end of the primitive ulnar anlage and lies further from the promontory than in earlier stages (fig. 31), as the result of an enlargement and elongation of the fenestræ which lie between its promontorial connections. The dorsal veno-lymphatic plexus, as already described, has been separated by fenestration from the dorso-lateral circumference of the promontory and primitive ulnar segment of the postcardinal, and its general phases of development can now be easily followed by comparing the preceding figures.

The anlage common to the primitive ulnar vein and the primitive ulnar veno-lymphatic is fully established and opens into the dorso-lateral circumference of the promontory about opposite the latter's point of confluence with the seventh dorsal somatic tributary (*7S*, fig. 42). The anlage of the primitive ulnar veno-lymphatic has not yet begun to separate from the anlage common to it and the primitive ulnar vein.

The anlage of the cephalic vein has made its appearance on the left side of this embryo as a small vessel which opens laterally into the promontory, dorsal to the anlage of the permanent external jugular vein.

This embryo shows most clearly that the veno-lymphatic area of the promontory has differentiated along its dorso-lateral circumference, leaving its dorso-medial aspect free for the entrance of the dorsal somatic tributaries (**4S, 5S, 6S** and **7S,** fig. 42). A similar dorso-lateral position with respect to the precardinal has already been described and emphasized as being characteristic of the veno-lymphatic derivatives of its dorsal tributaries (see figs. 33, 34, etc.).

In general, the relative position of the *dorso-lateral* veno-lymphatic (dorsal veno-lymphatic plexus and caudal division of ventral veno-lymphatic plexus) and the *dorso-medial* somatic (**4S, 5S, 6S**) elements with respect to each other and to the precardinal vein, is clearly shown in fig. 43, which is a photomicrograph of a transverse section of a 10 mm. cat embryo taken at a level just anterior to the jugular promontory (series 114, slide 4, section 27).

Series 102, 8.5^{mm} Embryo
Reconstruction of the left side
Lateral aspect, fig. 44 and
Medial aspect, fig. 45
Reconstruction of right side,
Lateral aspect, fig. 46

Although this embryo measures less than other members of this group, due probably to a greater degree of body and head curvature, it represents a more advanced stage of development, which bridges the interval between the intermediate and definitely established veno-lymphatic periods.

Reconstruction of Left Side, Lateral Aspect, Fig. 44.

The three primary divisions of the veno-lymphatic plexus are fully established.

With the exception of the element contributed by dorsal tributary **1** (**1VL**), which forms a dilated and fenestrated sac, connected with the precardinal, all of the components of the ventral division of the veno-lymphatic plexus have fused with one another to form a large and multi-fenestrated sac.

The cephalic division of the ventral plexus has been formed by tributary 1 (1VL) and from the area of the precardinal which receives dorsal tributaries 2 and 3 (2VL and 3VL), the latter two having retained their original precardinal terminations.

The caudal division of the ventral veno-lymphatic plexus is a capacious and multi-fenestrated sac which lies dorso-lateral to the precardinal and for the most part in front of the promontory. It is continuous in front with the anterior division of the ventral plexus and communicates with the promontory by two well-defined apertures: one, the smaller of the two, being situated near the promontorio-precardinal angle on the lateral surface (Tap *B*, fig. 44), and the other (Tap *D*, fig. 44) on the cephalic surface of the promontory in close association with the promontorial end of the dorsal somatic tributary 4 (4S, fig. 45).

This capacious sac which constitutes the caudal division of the ventral veno-lymphatic plexus has been formed as the result of a further development of a condition similar to that found in series 109 (fig. 30), series 2 (fig. 32) or series 19 (fig. 41), and has been derived either from a para-precardinal channel or from a para-precardinal channel in connection with a veno-lymphatic component derived from precardinal tributary 4.

Although we know, in general, the elements which may enter into the formation of the ventral plexus, on account of the variability of the process, it is extremely difficult to determine definitely in all cases of advanced stages exactly how its formation has been brought about. In view of the intimate relations which exist between this plexus and the first of the promontorial dorsal somatic tributaries (4S) which was derived from precardinal tributary 4, we are inclined to believe that in this case the veno-lymphatic component of the latter as well as the para-precardinal channel have entered into its formation.

The dorsal veno-lymphatic plexus is represented by a pouch-like fenestrated sac which was derived from the dorso-lateral aspect of the promontory. It projects forward, dorsal to the caudal division of the ventral veno-lymphatic plexus, and now communicates with the promontory by only a single narrow opening (Tap *C*, fig. 44) near the former's point of confluence with the

primitive ulnar vein. As mentioned above, in connection with the description of series 106 (fig. 40), this condition has been reached as the result of a separation of the sac from the anterior end of the anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic, and by the retention of only one of its primary promontorial points of connection. This single tap (Tap *C*, fig. 44) corresponds, approximately, in its position to that of the lymphatico-venous connection of later stages which is found at the jugulo-subclavian junction, while the more ventrally situated tap (Tap *B*) corresponds, approximately, to that met with at the junction of the external and internal jugular veins.

The thyro-cervical artery passes laterad close to the surface of the promontory, between the dorsal plexus and the caudal division of the ventral veno-lymphatic plexus.

This artery, from this stage on, is constantly encountered in this position and offers a uniform relationship to the components of the veno-lymphatic plexus. It thus constitutes an important landmark in differentiating in later stages and in the adult the parts of the united jugular lymph sac derived, respectively, from the dorsal plexus and caudal division of the ventral veno-lymphatic plexus.

The promontorial end of the dorsal veno-lymphatic plexus constitutes the subclavian approach of later stages and can always be identified by the constant relation which it holds to the ventrally situated thyro-cervical artery.

The anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic has separated into these two components. The former arches over the sixth spinal nerve (SP.N.VI) and opens into the dorso-lateral circumference of the promontory between the latter's point of confluence with the sixth and seventh dorsal somatic tributaries (6S and 7S, figs. 44 and 45). The primitive ulnar veno-lymphatic is a small vessel which runs parallel to the primitive ulnar vein. It has been completely differentiated from this vein except at its anterior end where indications of its former connection with the same are still evident. At a later stage it will completely separate from the primitive ulnar vein and, after joining the dorsal veno-lymphatic plexus, form a caudal extension of the latter.

Series 102, 8.5^{mm} Embryo
Reconstruction of left side,
Medial aspect, fig. 45

This view of the reconstruction shows three tiers of elongated fenestræ and deep depressions on the medial surface of the caudal division of the ventral veno-lymphatic plexus which appear to divide it into four parallel channels. Taken by itself this view of the reconstruction suggests that this portion of the plexus has been formed as the result of a fusion between four parallel vessels. Such is not the case, however, since the ontogenetic history of this plexus proves conclusively that these parallel vessels are secondarily formed as the intervals between the fenestral spans developed in the plexiform anlage of this structure.

The medial view of the reconstruction also shows the extensive and dilated character of the promontory and the relations which it holds to its dorsal somatic (**4S**, **5S**, **6S**, and **7S**, fig. 45) and veno-lymphatic structures (*Cf.* also fig. *43*).

Series 102, 8.5^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 46

As on the left side of this embryo the three primary divisions of the veno-lymphatic plexus are fully established on the right side.

I. Ventral Veno-Lymphatic Plexus

1. *Cephalic Division*

The precardinal dorsal tributary **1** (**1 VL**) is represented by a dilated, elongated and fenestrated sac which has retained its primary connection with the precardinal. It has not yet fused at its base with the adjacent veno-lymphatic components (**2 VL**, **3** (**VL**, and in this respect both sides of this embryo agree. In addition to its contribution, at its base, to the ventral plexus, this veno-lymphatic component of tributary **1** (**1 VL**) will, by its further dilation and caudal extension, fuse with the dorsal veno-

lymphatic plexus and thus contribute to the forward extension of the latter. If, in the meantime, it still retains its primary connection with the precardinal, this connection may serve as a portal through which the blood is evacuated from the plexus into the systemic veins (Tap of evacuation).

The distinctive element of the cephalic division of the ventral plexus in this region is represented by a capacious sac which has shifted to the lateral aspect of the precardinal and which communicates with the same by a single opening (Tap *A'*). This sac occupies an area of the precardinal which formerly received the dorsal tributaries 2 and 3. The primary connection of one of these two tributaries (probably 2) with the precardinal has undoubtedly persisted as the opening through which the sac now communicates with the vein, while the sac itself has probably been formed through a fusion of the veno-lymphatic components of these two tributaries, possibly in conjunction with an element derived from the plexiform dorso-lateral circumference of the main channel of the precardinal vein.

The cephalic portion of the ventral division of the veno-lymphatic plexus is not always developed to the extent shown in the present embryo but, when present, its origin can always be traced back to the veno-lymphatic components of the precardinal tributaries 1, 2 and 3, to the area of the precardinal which receives these tributaries, or, to both of these sources.

2. *Caudal Division.*

The caudal division of the ventral veno-lymphatic plexus, which has undoubtedly been derived from the veno-lymphatic component of precardinal dorsal tributary 4 and its related paraprecardinal channel, forms an extensive sac with three elongated cephalic processes. The sac has shifted laterad and caudad on to the lateral aspect of the precardinal and promontory, a position which becomes more pronounced in later stages. It communicates laterally (fig. 46) with the precardinal near the promontorio-precardinal angle (Tap *B'*) and with the promontory slightly caudal to this point (Tap *B*).

The dorsal somatic component of tributary 4 (4S) is entirely detached from the veno-lymphatic plexus, and enters, as the first of the somatic series, the dorso-medial aspect of the promontory.

II. Dorsal Veno-Lymphatic Plexus.

This is a much dilated and fenestrated sac which lies dorso-lateral to the promontory, and which has been derived from the same. It extends forward for some distance in front of the promontory and communicates with the latter by a narrow channel which opens into the promontorial end of the primitive ulnar vein (Tap *C*). The presence of a single point of communication between the dorsal plexus and the promontory at the primitive ulnar entrance corresponds to the conditions already described for the left side of this embryo (Tap *C*, fig. 44). The relations of the the dorsal plexus to the thyo-cervical artery are also the same and need, therefore, no further mention.

The primitive ulnar vein makes a distinct arch in its terminal portion and receives a long cephalic tributary which follows the dorsal border of the dorsal veno-lymphatic plexus. The primitive ulnar veno-lymphatic was not fully reconstructed on this side of the embryo but presents about the same conditions as on the left side.

In addition to the three primary veno-lymphatic plexuses already described, a number of detached veno-lymphatic elements are met with on the right side of this embryo. These are four in number and are labelled *VL'* in fig. 46. The significance and subsequent fate of these detached veno-lymphatics has already been discussed on a preceding page (page 259) and therefore need not be further considered here.

The medial aspect of this reconstruction, which is not figured, like that of the left side (fig. 45), offers a typical instance of the serial arrangment of dorsal somatic tributaries, beginning with **4S,** which enter the dorso-medial aspect of the promontory.

As this embryo serves as a connecting link between the early and later veno-lymphatic stages, it may be well to summarize the principal features which it illustrates.

1. Gradual reduction of the multiple early veno-lymphatico-venous connections, but retention, in some cases, of the primary precardinal connections of tributaries 1, 2, and 3.

2. The gradual establishment of two definite points of communication between the promontory and the dorsal (Tap *C*) and ventral (Tap *B*) veno-lymphatic plexuses, respectively, which are usually the last to be retained during the separation process and which correspond, approximately, in their position to the points where the jugular lymph sac taps the venous system in the adult.

3. Clear differentiation of the veno-lymphatic plexus into three primary divisions and the beginning of a fusion between the two divisions of the ventral plexus.

4. Complete separation of the dorsal and ventral veno-lymphatic plexuses by a clear interval which serves for the transit of the thyro-cervical artery.

5. Separation of the dorsal plexus from the anterior end of the anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic.

6. Separation from this common anlage of a small parallel channel which forms the primitive ulnar veno-lymphatic. This veno-lymphatic joins the dorsal plexus and remains connected with the caudal end of the same after the primitive ulnar vein has been replaced in the drainage of the anterior limb by the definite subclavian vein.

7. The presence of a number of detached veno-lymphatics which subsequently fuse with one another and with the three primary divisions of the plexus in establishing the single veno-lymphatic sac of later stages.

8. The definite establishment of the anlage of the cephalic vein.

TWO EMBRYOS ILLUSTRATING THE LATERAL DISPLACEMENT OF CERTAIN VENO-LYMPHATICS WHICH ARE SECONDARILY SEPARATED BY FENESTRATION FROM THE CAUDAL DIVISION OF THE VENTRAL VENO-LYMPHATIC PLEXUS.

Series 112, 10^{mm} Embryo
Series 113, 10^{mm} Embryo

As the result of an excessive retiform development in the caudal division of the ventral veno-lymphatic plexus it frequently,

though not invariably, happens that a portion of the same becomes partially or wholly detached and is displaced to the lateral surface of the precardinal vein and the promontory.

This feature of development appears to be of sufficient importance to warrant a careful consideration, as it further emphasizes the variable character of the process of fenestration, as well as the variable form which may be assumed by this division of the ventral plexus as the result of this process.

The two embryos in question illustrate exceedingly well the partial separation of a portion of the ventral plexus and its displacement to the lateral surface of the promontory and precardinal vein. They also give us the clue as to the origin of the independent and completely isolated veno-lymphatic sacs or spaces which are met with in certain cases on the lateral surface of the promontory and precardinal vein (fig. 50).

Series 112, 10^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 47

The Caudal Division of the Ventral Veno-Lymphatic Plexus.

The caudal division of the ventral veno-lymphatic plexus in this embryo (fig. 47) is a less condensed and compact structure than in the 8.5 mm. embryo (figs. 44 and 46), just considered. This does not necessarily mean that it is less advanced in development than in the 8.5 mm. embryo (series 102), but rather that the plexiform character has been longer retained in it than in the case of the latter, at a stage which precedes the general amalgamation of all the veno-lymphatics to form a single common sac. In this embryo (fig. 47) the caudal division of the ventral veno-lymphatic plexus consist of three distinct and parallel channels.

A large dorsal channel, continuous with the dorsal veno-lymphatic plexus, which joins anteriorly the ventral of the three channels by two transverse vessels; an intermediate smaller channel which opens into the lateral surface of the promontory at Tap B' and into the dorsal surface of the ventral channel, and of a large ventral channel lying upon the lateral surface of the pre-

cardinal and promontory, which communicates with the latter at Tap *B* and at its cephalic extremity with the precardinal vein (Tap *X*).

On comparing the caudal division of the ventral plexus in the 10 mm. (fig. 47) and 8.5 mm. (figs. 44 and 45) embryos, it is evident that a further confluence of the fenestræ in the caudal division of the ventral plexus of the 8.5 mm. embryo (fig. 45) would produce a series of parallel and partially separated channels which hold the same relations to the precardinal and promontory, as do the three parallel channels in the 10 mm. embryo (fig. 47). It is also clear, considering our knowledge of the ventral plexus, in general, that the ventral and intermediate of the three channels in the 10 mm. embryo (fig. 47) must have been separated from that portion of the veno-lymphatic complex of tributary **4** and its associated para-precardinal channel, which has been contributed by the para-precardinal channel.

A comparison of the 10 mm. (fig. 47) and 7 mm. (series 138, figs. 33 and 35) embryos also gives us a clue, at this late stage of development, concerning the origin of the most dorsal of the three channels which compose the caudal division of the ventral veno-lymphatic plexus in the 10 mm. embryo (fig. 47).

The Dorsal Division of the Veno-Lymphatic Plexus.

This embryo is characterized by extensive development in the area of the dorsal division of the veno-lymphatic plexus, and by the complete separation of the same from the systemic veins except by means of its connection with the latter through the caudal division of the ventral plexus. It therefore prepares the way for the consideration of subsequent stages in which the entire veno-lymphatic plexus, as a closed and empty sac, appears to separate temporarily from the systemic veins.

The possibilities of forward extension of the dorsal veno-lymphatic plexus are represented here by a number of detached and closed sacs (*VL'* in fig. 47), lying in the course of its secondary forward growth, which subsequently by fusion with it and with the veno-lymphatics of the cephalic division of the ventral plexus, will

complete the secondary dorsal veno-lymphatic arch of later stages (fig. 51, series 77).

The primitive ulnar veno-lymphatic has become detached from the vein from which it arose by condensation of the surrounding capillary network, and now enters the caudal extremity of the dorsal veno-lymphatic plexus.

The relations of the thyro-cervical artery to the dorsal veno-lymphatic plexus and to its ventro-caudal extension, the subclavian approach, are the same as in the preceding embryo (series 102, fig. 44). The jugular approach is represented by that portion of the ventral plexus which opens into the promontory at the two points labelled Tap *B* and Tap *B'* in fig. 47.

The Cephalic Division of the Ventral Veno-Lymphatic Plexus.

The veno-lymphatic components of tributaries 1, 2 and 3 which constitute the anlages of this division of the ventral plexus (**1**, **2** and **3VL**, fig. 47), although modified by retiform fenestration, still retain their individuality in this embryo, a condition which may or may not obtain up to a late stage of development.

The embryo offers a good concrete example of the following general phases of veno-lymphatic development:

1. Unequal development of the two main veno-lymphatic plexuses at any given stage.

2. Beginning separation of the veno-lymphatic sac, formed by further condensation of the plexuses from the definite venous channels.

3. The formation of isolated and completely closed sacs or spaces, detached both from the veins and from the main veno-lymphatic plexuses, which are subsequently incorporated in the jugular lymph sac.

4. The genesis of the secondary dorsal veno-lymphatic bridge or arch, which in certain subsequent stages connects the cephalic end of the dorsal plexus with the anterior veno-lymphatic channels of the ventral division. This structure which, e. g., appears in its characteristic development in series 77 and 101 (figs. 51 and 50), is apparently developed in different individuals to an unequal degree and in one of the following ways:

(*a*) Cephalic extension of the dorsal veno-lymphatic channel to union with the cephalic division of the ventral plexus.

(*b*) Caudal growth of part of the cephalic division of the ventral plexus until union with the dorsal plexus has been effected.

(*c*) Development of intermediate detached areas of veno-lymphatic character, which, isolated at first, subsequently unite with both the dorsal veno-lymphatic plexus and with the cephalic division of the ventral plexus, and thus effect the junction of the secondary arch with the primary veno-lymphatic elements.

Series 113, 10^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 48

This embryo is characterized by multiple discrete veno-lymphatic development along the entire dorso-lateral circumference of the precardinal and promontory, resulting in the formation of numerous early communication between the veno-lymphatic plexuses and the main venous channel. It also shows marked displacement of a portion of the caudal division of the ventral veno-lymphatic plexus from the dorsal to the lateral aspect of the promontory.

The Caudal Division of the Ventral Veno-lymphatic Plexus.

This division of the ventral plexus is composed of a number of elements, with separate entrances into the precardinal and promontory.

These elements group themselves into two main areas, anterior and posterior, each of which contains subordinate components.

1. Anterior Area of the caudal division of the ventral plexus.

The anterior portion of the caudal division of the ventral plexus is separated, as in the preceding series 112, into two parallel elements, a ventral and a dorsal (fig. 48, *V* and *D*), which are joined by a narrow curved piece. The former element (*V*), representing

the para-precardinal channel, is displaced ventro-caudad from the dorsal to the lateral surface of the precardinal and promontory, forming a dilated sac which communicates with the promontory at three points (area of Tap *B*, fig. 48), while in series 112 (fig. 47) the promontorial end of this correspondingly displaced portion of the ventral plexus consists of two parallel channels which open independently of each other into the lateral surface of the promontory (Tap *B*, and *B'*, fig. 47). The difference in form, as well as the difference in the number of promontorial communications encountered in the two embryos, is due to varying phases of capillary condensation and amalgamation in the development of these partially detached portions of the ventral plexus. A comparison of figs. 44, 45, 47, and 48 clearly shows the transitional stages leading up to the conditions presented by series 113 (fig. 48).

The second element (*D*) composing this anterior portion of the caudal division of the ventral plexus consists of a channel, lying cephalad of the promontory and parallel to the precardinal vein. It communicates with the latter at two points, and in addition, as above stated, is connected by a narrow curved channel with the first expanded sac-like element (*V*) on the lateral surface of the promontory. It, including its dorsal branches (**4VL** in fig. 48), has been derived from the original complex formed by precardinal tributary **4**, and the para-precardinal channel.

The original connection of element *D* with the promontory is, however, still represented by the most anterior of a series (*E'*, *E''*, and *E'''*) of small veno-lymphatic channels (fig. 48), which form together the posterior or caudal portion of the caudal division of the ventral plexus. This small veno-lymphatic (fig. 48, *E'*) opens into the promontory in line with the elements *D* of the anterior portion of the caudal division, and probably has become secondarily detached from the same in the course of development. Compare fig. 48 with fig. 33, in which a similar separation of the ventral plexus from the promontory at *B* has taken place, and with fig. 42 in which the caudal division of the ventral plexus is alone represented by a small promontorial veno-lymphatic, the remainder of the complex of tributary **4** having developed into dorsal somatic tributary **4S**.

2. Posterior Area of the caudal division of the ventral plexus.

In this embryo, as clearly indicated by the course of the thyro-cervical artery, the caudal division of the ventral plexus receives an addition of plexiform veno-lymphatic anlages (fig. 48, *E'*, *E''*, *E'''*) which communicate with the lateral surface of the promontory by five openings. This veno-lymphatic plexus intervenes between the caudal termination of the two elements (*V* and *D*) forming the anterior portion of the caudal division of the ventral plexus, and the dorsal veno-lymphatic plexus. They are undoubtedly destined to fuse with each other and with the remaining components of the caudal division. After this fusion has occurred the thyro-cervical artery will then occupy its typical and invariable position, arching over the shoulder of the promontory between the dorsal veno-lymphatic plexus and the united caudal division of the ventral plexus.

The Dorsal Veno-Lymphatic Plexus.

The dorsal veno-lymphatic plexus of this embryo (fig. 48) is still incompletely separated from the promontory. It forms a sac-like fenestrated structure which opens into the distal portion of the promontory (fig. 48, Tap *C*), as well as into the primitive ulnar arch.

The Cephalic Division of the Ventral Veno-Lymphatic Plexus.

The veno-lymphatic components of precardinal tributaries **1**, **2** and **3** (fig. 48, **1VL**, **2VL** **3VL**), which will unite to form the cephalic division of the ventral plexus, are still individually distinct; **1VL** and **2VL** are united by a narrow channel, **3VL** is still separate, partially hidden in the figure by the second spinal nerve (fig. 48, *SP.N.II*).

In addition to the separation of the caudal division of the ventral plexus into separate components and the displacement of one of these components from the dorsal to the lateral surface of the promontory, this embryo is characterized by the large number of discrete connections which still persist between the veno-lymphatics and the systemic veins. Beginning with precardinal tribu-

tary **1** (fig. 48, **1VL**) as many as sixteen of these connections are met with along the precardinal vein and the promontory.

FULLY DEVELOPED VENO-LYMPHATIC STAGE.

Series 101, 10^{mm} Embryo
Series 77, 11^{mm} Embryo

Series 101 is characterized by enormous cephalic extension of the dorsal veno-lymphatic plexus, which, as a minutely fenestrated multilocular sac, extends forward to the level of the cephalic arch of the precardinal.

The two main divisions of the ventral veno-lymphatic plexus are well differentiated, but still entirely separate from each other and from the dorsal plexus.

Series 101, 10^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 49

The group of veno-lymphatics which constitutes the cephalic division of the ventral veno-lymphatic plexus, derived from the precardinal tributaries **1**, **2** and **3**, is represented by two independent dilated sacs both of which still retain a connection with the precardinal. The united trunk of the first and second spinal nerves (*SP.N.I* and *II*) passes ventro-laterad between the cephalic of these two sacs and the precardinal, while the third nerve (*SP.N.III*) passes between the caudal sac and the dorsal veno-lymphatic arch.

The caudal division of the ventral veno-lymphatic plexus is an irregular-shaped sac which lies upon the lateral surface of the precardinal and promontory. It communicates with the venous system at only one point (Tap *B*), by a wide orifice situated on the lateral surface of the promontory near the promontorio-precardinal angle. This sac must be regarded as representing a further development of the para-precardinal channel and the associated veno-lymphatic component of precardinal tributary **4**.

In conformity with a greater degree of veno-lymphatic development, the caudal division of the ventral plexus has given up its

multiple connections with the precardinal and promontory and retained only one which corresponds, approximately, to the primary point of connection of the para-precardinal channel with the promontory. This point of connection, as mentioned above. is one of the last to be given up, and also corresponds, approximately, in its position to that of the common jugular tap in the adult (*cf.* fig. 1, left side).

Just dorsal to the entrance of the cephalic vein into the lateral surface of the promontory, the thyro-cervical artery passes ventrolaterad in the interval between the ventral and dorsal divisions of the veno-lymphatic plexus.

The dorsal veno-lymphatic plexus is now a very extensive structure which extends from the region of the promontory, as far forward as the level of the cephalic arch of the precardinal where it ends blindly. In the region of the promontory the dorsal plexus is still broadly confluent with the anlage common to the primitive ulnar vein and primitive ulnar veno-lymphatic which has not as yet separated into its two components. The line of cleavage along which the separation will take place, however, is clearly indicated by a row of fenestrae which will subsequently become confluent. (Compare with fig. 47).

Just in front of its connection with the primitive ulnar arch the dorsal plexus gives off a ventrally directed tongue-shaped process (subclavian approach) which communicates with the promontory by a single opening (Tap *C*) and which lies near the entrance into the promontory of the primitive ulnar vein. This point of communication with the promontory corresponds in all respects to that previously described for this plexus in connection with series 102 (Tap *C*, figs. 44 and 46).

This extensive development of the dorsal veno-lymphatic plexus is the result of a forward growth of the redundant plexiform area of the promontory and convexity of the primitive ulnar arch and inclusion within the same of detached veno-lymphatics which have been derived from the dorsal tributaries of the precardinal.

The fifth spinal nerve (*SP.N.V*) penetrates the dorsal plexus, while the sixth (*SP.N.VI*), and following spinal nerves in this

region pass between the primitive ulnar anlage and the main systemic venous channel.

Series 101, 10^{mm} Embryo
Reconstruction of right side,
Lateral aspect, fig. 50

The right side of this embryo (fig. 50) offers an interesting comparison with the veno-lymphatic development found on the left side (fig. 49).

A. Dorsal Veno-Lymphatic Plexus.

The dorsal sac presents the same forward extension and multifenestrated appearance as that on the left side, but its relations to the promontory and to the straight segment of the precardinal differ in a number of important features from those of the opposite side.

1. On the right side (fig. 50) the dorsal plexus is plainly separated into two portions, one derived from the promontory proper, the other from the arch of the primitive ulnar vein.

The posterior and smaller part of the general dorsal veno-lymphatic plexus is not connected, as it is on the left side, with the larger anterior part.

2. The anterior end of the dorsal plexus has established a connection, near the level of the cephalic arch, with the straight segment of the precardinal (Anterior Tap of Evacuation). This connection results from the fusion between the anterior end of the dorsal plexus and the veno-lymphatic component of a dorsal tributary of the straight segment of the precardinal which has retained its primary connection with the latter, or, which, in case of early multiple precardinal terminals, has continued to develop one of these, thus accounting for the difference in the level of the first precardinal veno-lymphatic tap observed on the two sides of this embryo in reference to the level of the spinal nerves.

On the left side the abandonment of the early precardinal terminal of tributary 1, together with the retention of the terminals of tributaries 2 and 3, would produce the relations to the segmental nerves found on this side of the embryo, while the relations of the

nerves to the veins and veno-lymphatics of the right side suggest that here the terminal of tributary 1 has been retained, while tributaries 2 and 3 have lost their precardinal connections. Thus on the left side the common trunk of the first and second cervical nerves comes to lie cephalad of all veno-lymphatic terminals into the precardinal, while on the right side this trunk is placed caudad of the first veno-lymphatic precardinal tap.

3. On the left side (fig. 49) the extension cephalad of the dorsal plexus involves both the portion derived from the promontory and that furnished by the primitive ulnar arch, both components uniting in the plexiform network of the dorsal veno-lymphatic arch. On the right side (fig. 50) the primitive ulnar arch yields a relatively small plexiform veno-lymphatic cephalic prolongation, which, while undoubtedly destined to unite eventually with the other components of the dorsal veno-lymphatic arch to form its caudal extension is, in this stage, still separate and discrete. The arch is in this instance evidently produced by cephalic extension of that portion of the dorsal plexus, which was originally derived from the promontory, but which, in establishing a secondary anterior connection with one of the veno-lymphatic compounds of the cephalic division of the ventral plexus, *has given up its original promontorial connection in the area of Tap C* (*cf.* fig. 44).

4. On the right side (fig. 50) the promontorial portion of the dorsal plexus has given up its original connection with the promontory, still present on the left side, and now ends in this region in a caudal blind prolongation which lies dorsal to the thyrocervical artery, and constitutes that portion of the future lymph sac which we have termed the subclavian approach.

5. This right side of this embryo (fig. 50) illustrates an important phase in veno-lymphatic development, which, in a more advanced condition, is found in series 78 (fig. 57). In embryos ranging between 10 and 12.5 mm. the dorsal veno-lymphatic plexus makes a secondary, although temporary, connection with the anterior part of the straight segment of the precardinal, and in doing so, often gives up its original promontorial connection (series 78, fig. 57). The secondarily acquired anterior connection with the precardinal vein apparently serves for the evacuation of

the blood-contents from the plexiform sac into the systemic veins. After the process of evacuation is completed *this precardinal connection is abandoned.* It is not possible to state definitely that this anterior tap of evacuation invariably develops in all cat embryos, but we can state that it occurs with frequency in embryos ranging between 10.5 and 12.5. mm. in length. (*Cf.* p. 291.)

B. Ventral Veno-Lymphatic Plexus.

1. *Cephalic division*

This portion of the ventral plexus (fig. 50) is represented by two veno-lymphatics, one of which has fused with the anterior prolongation of the dorsal veno-lymphatic plexus while the other, a small dilated sac, fenestrated at its base and still connected with the precardinal, lies directly caudal to the anterior tap of evacuation.

2. *Caudal division.*

A considerable difference is met with on opposite sides of this embryo concerning the make-up of this portion of the ventral plexus. On the left side (fig. 49) it consists of a single, irregular sac which communicates with the promontory by a single opening (Tap B, fig. 49). On the right side (fig. 50) it is composed of two entirely detached and independent sacs and of a portion which still communicates with the systemic veins at three points.

The two detached and independent sacs are completely closed and, as far as we can determine under a high magnification, do not communicate with the venous system. The anterior and larger of the two sacs is irregular in form and lies on the lateral surface of the precardinal and promontory near the promontorio-precardinal angle. In its form and position it closely resembles the caudal division of the ventral plexus on the left side of the embryo (fig. 49). It contains no blood corpuscles and from a histological standpoint cannot be distinguished from a typical lymphatic structure. The other closed sac lies upon the lateral surface of the promontory, slightly dorsal and caudal to the first and between the latter and the anlage of the cephalic vein. This sac still contains a large number of blood corpuscles.

The remaining portion of this plexus presents an appearance which has been shown to be typical for this structure in a number of the preceding stages (e. g. fig. 48). It consists of a vessel running parallel to the precardinal, somewhat arched anteriorily, which opens into the precardinal at two points (Tap X and Tap X', fig. 50) and into the lateral surface of the promontory by a single orifice (Tap B). Opposite its point of communication with the precardinal at Tap X', it is connected by means of a very narrow stalk with a dilated oval blind sac whose caudal extremity approaches the promontory just in front of the thyro-cervical artery. This expanded appendage to the caudal portion of the ventral veno-lymphatic plexus is an important feature of development and appears to represent a condition frequently obtained by this plexus previous to its subsequent amalgamation with the dorsal plexus and the cephalic division of the ventral veno-lymphatic plexus to form a common sac (*cf.* fig. 14).

Although exactly the same conditions seldom prevail in any two embryos, it is not difficult to interpret the origin of the caudal division of the ventral plexus in the present instance on the basis of a further development of the conditions shown in series 19 (fig. 41, 9 mm. embryo).

The channel of the caudal division of the ventral veno-lymphatic plexus which, in the 10 mm. embryo (fig. 50, series 101) runs parallel to the precardinal and taps the systemic veins at three points (Tap X, X' and B), has its homologue in the 9 mm. embryo (fig. 41, series 19) in the para-precardinal channel and the element L which has been separated from the latter, while the oval blind sac in series 101 (fig. 50) which is connected with this parallel channel by a narrow stalk has been derived from the veno-lymphatic component of precardinal tributary 4 in series 19 (**4VL,** fig. 41). On the other hand, the two detached and closed sacs in series 101 (fig. 50) are the secondary products of the original para-precardinal plexus (fig. 41, see also fig. 31) which have become separated from the same by an increase in the process of condensation, as in figs. 47 and 48.

The transformation of the complex of tributary 4 and the para-precardinal channel into two or more parallel channels is a feature

of common occurrence, and is well illustrated by series 102 (fig. 45) and by series 112 (fig. 47) in which two such channels are met with opening into the lateral surface of the promontory at Tap *B*. In the latter series, if these two channels were to separate completely from each other and from the remaining portion of the ventral plexus, they would occupy the same relative position with respect to each other and to the precardinal vein and promontory as do the two closed sacs in series 101 (fig. 50).

Series 77, 11^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 51 and
Reconstruction of right side,
Lateral aspect, fig. 52

This 11 mm. embryo offers an exceedingly interesting and suggestive comparison with the 10 mm. embryo just considered (figs. 49 and 50), since it further emphasizes two principal features of development first noted in the latter:—(1) the forward extension of the dorsal veno-lymphatic plexus and the establishment of an anterior tap of evacuation and (2) the beginning of the amalgamation of the three primary divisions of the veno-lymphatic plexus to form a common single veno-lymphatic sac.

On both sides of the embryo, (figs. 51 and 52) an anterior tap of evacuation has been established at the level of the cephalic arch, between the anterior end of the dorsal veno-lymphatic arch and the straight segment of the precardinal. As mentioned above, we have every reason to believe that this communication, secondarily established, between the dorsal arch and the precardinal, represents a normal phase of development and serves as the main, if not the only exit, in some cases, by which the blood is evacuated from the veno-lymphatic sac into the systemic veins.

Further, on both sides of this embryo (figs. 51 and 52), the cephalic division of the ventral veno-lymphatic plexus has become incorporated by fusion with the anterior end of the dorsal plexus so that this portion of the ventral plexus no longer appears as a separate structure.

On the left side (fig. 51) the dorsal veno-lymphatic plexus com-

municates with the lateral surface of the promontory, through the subclavian approach, by a single opening (Tap *C*). This point of communication lies between the openings into the promontory of the cephalic and primitive ulnar veins and corresponds in its position and relations to a promontorial communication of the dorsal plexus observed in series 102 and 101 (Tap *C*, figs. 44, 46, and 49). In addition to this promontorial communication the dorsal plexus still retains a connection with the primitive ulnar vein and also communicates with the caudal division of the ventral veno-lymphatic plexus by a narrow transverse channel. Caudally the dorsal plexus of the left side (fig. 51) receives the primitive ulnar veno-lymphatic which is now completely separated from the primitive ulnar vein. The latter, however, has retained its original connection with the dorsal plexus, but has differentiated from the postcardinal and promontory except at its anterior end where it arches over the sixth spinal nerve (*SP.N. VI*, fig. 51).

On the left side (fig. 51) the caudal division of the ventral veno-lymphatic plexus consists of a single sac which, as mentioned above, communicates by a narrow channel with the dorsal plexus and by a single opening (Tap *B*) with the lateral surface of the promontory near the union of the latter with the cephalic vein. This point of communication with the promontory (Tap *B*) corresponds, approximately, in its position and relations to the promontorial communication of the ventral plexus observed in series 102, 112 and 101 (Tap *B*, figs. 44, 46, 47, 49 and 50), and the portion of the ventral plexus directly involved in the communication constitutes that portion of the future jugular lymph sac which we have termed the "*jugular approach.*" *By tracing back its relations to the promontory in the above mentioned series, it will be seen that the so-called 'jugular approach' has its origin in the para-precardinal channel of earlier stages and that the communication at Tap B represents one of the persistent openings by which the para-precardinal channel and its associated veno-lymphatic component of precardinal tributary* 4 *communicates with the promontory.*

The caudal division of the ventral veno-lymphatic plexus, as mentioned above, has assumed a characteristic form which appears to precede its fusion with the dorsal plexus. It is composed of a

ventrally situated elongated portion which lies along the lateral surface of the precardinal and promontory and of an expanded, oval dorsal portion which is flexed mesad so that it lies directly upon the dorsal surface of the precardinal, just in front of the promontory (figs. 14 and 20). The promontory, however, does not form a prominent swelling of the main venous channel on the left side of this embryo.

On the right side of this embryo (fig. 52), in addition to the anterior tap of evacuation, the dorsal veno-lymphatic plexus communicates with the systemic veins at three points, all of which are situated on the dorso-lateral surface of the promontory. The most anterior of these three points of communication (Tap *C*), made through the subclavian approach, lies dorsal to the cephalic vein and caudal to the thyro-cervical artery and in this respect corresponds to the Tap *C* observed on the opposite side of the embryo (Tap *C*, fig. 51). The two points of communication caudal to Tap *C* on the right side (fig. 52) represent a condition in which the process of separation of the dorsal plexus from the promontory is less complete, owing to the establishment of secondary connections representing the anlage of the subclavian vein terminal. The primitive ulnar veno-lymphatic is fully established on the right side but, as it was not reconstructed, has not been indicated in the figure.

In addition to its connection with the systemic veins, the dorsal veno-lymphatic plexus also communicates, by two narrow transverse channels, with the caudal division of the ventral plexus. These two connections, as well as the single one of the opposite side, must be regarded as secondary communications which have been established between the dorsal and ventral plexus preliminary to a complete fusion between the two. The composition of the caudal division of the ventral plexus is essentially the same as on the left side. It consists of an elongated portion, which lies along the lateral surface of the precardinal and promontory, and communicates with the latter (Tap *B*) near the entrance into the promontory of the cephalic vein, and of a dorsal oval, expanded portion which is flexed mesad so that it lies upon the dorsal surface of the precardinal, just in front of the promontory. The promontory is

a prominent structure on the right side, its outline being indicated in the figure by a broken line.

The relation of the first five spinal nerves to the veno-lymphatic plexuses is indicated in the reconstruction of the right side (fig. 52). The first and second (*SP. N. I* and *II*), third (*SP. N. III*) and fourth (*SP. N. IV*) nerves pass ventro-laterad between the cephalic and caudal divisions of the ventral veno-lymphatic plexus, and the fifth (*SP. N. V*) nerve passes directly through the dorsal plexus. The sixth spinal nerve (*SP. N. VI*, fig. 51) lies behind the dorsal plexus and arches ventro-laterad under the primitive ulnar vein.

The thyro-cervical artery, as in the preceding stage, lies ventral to the subclavian approach and between the dorsal and ventral divisions of the veno-lymphatic plexus.

These relations of the spinal nerves are retained in later stages after the primary divisions of the veno-lymphatic plexus have fused to form a common sac. Subsequently, after the completion of the veno-lymphatic process, the resulting jugular lymph sac divides at its anterior extremity (fig. 17), thus freeing the nerves, which then come to pass, first between the dorsal portion of the sac and the internal jugular vein, and, finally, entirely dorsal to the sac. The relation of the thyro-cervical artery to the lymph sac is, however, retained throughout and persists in the adult.

In view of the importance of the two embryos, which constitute this group (series 101 and 77), since they represent a stage of development which immediately precedes the establishment of the jugular lymph sacs, it may be well to tabulate the principal features which they illustrate, as follows:

Series 101, 10^{mm} Embryo
Figs. 49 and 50

1. This embryo furnishes an excellent example of the three main divisions of the veno-lymphatic plexus, fully developed, but still quite distinct from each other.

2. The extensive forward growth of the dorsal veno-lymphatic plexus and the establishment, on the right side, of an anterior

tap of evacuation through its union with a dorsal tributary of the precardinal.

3. The gradual reduction in the number of early multiple connections which exist between the veno-lymphatic plexus and the veins.

4. The complete separation of the dorsal plexus from the promontory on the right side and the persistence of a single point of connection on the left through the subclavian approach (Tap *C*).

5. The persistence of a single point of connection between the caudal division of the ventral veno-lymphatic plexus and the promontory on both sides of the embryo (Tap *B*), this representing the primary promontorial connection of the para-precardinal channel and its associated veno-lymphatic component of precardinal tributary 4.

6. The presence of two closed sacs on the right side, one without blood corpuscles, which have become detached from the para-precardinal channel and its associated veno-lymphatic component of precardinal tributary 4.

Series 77, 11^{mm} Embryo
Figs. 51 and 52

1. Forward extension of the dorsal veno-lymphatic plexus and the establishment on both sides of the embryo of an anterior tap of evacuation.

2. Fusion of cephalic division of ventral veno-lymphatic plexus with anterior end of dorsal plexus.

3. With the exception of the anterior tap of evacuation no communication exists between the straight segment of the precardinal and the veno-lymphatic plexus.

4. Two points of communication between the general veno-lymphatic plexus and the promontory are present which, in the entire series of embryos examined, appear the last to be given up, as well as the most constant in character; one is with the dorsal veno-lymphatic plexus through the subclavian approach (Tap *C*), the other with the caudal division of the ventral plexus through the jugular approach (Tap *B*). These two points of communica-

tion between the promontory and the veno-lymphatic plexus correspond, approximately, to the two points at which in later stages the jugular lymph sac taps the venous system (see fig. 3 of adult).

5. The embryo represents a stage just preceding the complete fusion of the dorsal and ventral plexus to form a common sac.

6. The caudal portion of the ventral plexus has assumed a characteristic form which appears to precede this fusion, its dorsal, dilated oval portion being flexed mesad so that it lies upon the dorsal surface of the precardinal just in front of the promontory, while its ventral, elongated segment follows the lateral surface of the promontory and precardinal, preparatory to its fusion with the anterior end of the dorsal plexus, which, in this case, also includes the cephalic division of the ventral plexus.

7. Relation of spinal nerves to the veno-lymphatic plexus to be compared with relation of these nerves to the jugular lymph sacs of later stages and of the adult.

8. Constant relations of thyro-cervical artery to the subclavian approach, to the veno-lymphatico-venous connection of the dorsal plexus at Tap *C*, and to the interval between the dorsal veno-lymphatic plexus and the caudal division of the ventral plexus.

PRE-LYMPHATIC STAGE.

Stage of Evacuation of blood Contents of Earlier Veno-lymphatic Plexus, and Apparent Complete Temporary Separation of the Empty Lymphatic Sac from the Permanent Venous Blood Channels.

Series 78, 12^{mm} Embryo
Series 474, 10.7^{mm} Embryo, Harvard Embryological Collection

We have already stated previously that no hard and fast lines can be drawn between the veno-lymphatic and definite lymphatic stages as far as the development of the jugular lymph sac is concerned. It is convenient, however, to define certain derivatives of the venous system, constituting the anlages of the jugular lymph sacs, as "veno-lymphatics," as long as these derivations are still filled

with blood and remain in free communication with the veins. Between this veno-lymphatic stage and the stage of final organization of the lymph sacs as definite portions of the entire lymphatic system, intervenes a very short period which we have designated as the *pre-lymphatic stage*. In this stage the venous derivatives appear fused together to form a single sac which then undergoes the following successive changes;

1 The blood contents of the sac are emptied in many cases into the systemic vein through a large connection, secondarily established, between the anterior end of the common sac and the precardinal vein (Anterior tap of evacuation). Except for this anterior connection between the systemic veins and the sac, the latter is distinctly lymphatic in character, and, from a histological standpoint, cannot be distinguished from the lymph sac of much later stages. It is possible that in certain embryos the promontorial connections serve as posterior taps of evacuation, in which case the anterior tap is not established.

2 After the sac has become emptied of blood contents the anterior tap of evacuation closes rapidly, and the sac, according to our observation, separates for a short time entirely from the venous system. A few doubtful points of apparent communication observed in some instances are very likely artifacts. In any case they differ entirely in character and appearance both from the earlier connections of the veno-lymphatic plexus with the veins, and from the later typical lymphatico-venous taps found after the adult conditions have been established.

Although in this pre-lymphatic stage no definite promontorial connection between the sac and the veins can be determined, the sac sends out two processes which approach and almost reach the two points which in the adult constitute the area of lymphatico-venous connections. These two points correspond, respectively, to those at which in the earlier stages the dorsal and ventral veno-lymphatic plexuses opened into the jugular promontory. (Compare fig. 16 with figs. 14, 15 and 17). It is difficult to account on physiological grounds for this apparent temporary separation of the sac from the veins. Since the permanent connections of the lymphatic and venous system take place apparently at the iden-

tical points occupied in the earlier stages by the terminals of the ventral and dorsal veno-lymphatic plexuses, it appears much more reasonable to assume a continuity of the channels throughout development. The picture afforded by the proper stages is, however, so definite and conclusive, that it becomes necessary to accept this separation as a normal feature of the pre-lymphatic stage, and as differentiating it sharply both from the earlier veno-lymphatic condition and from the later stage in which the definite secondary lymphatico-venous taps are established.

Series 78, 12^{mm} Embryo
Reconstruction of left side,
Lateral aspect, fig. 57

The left side of this embryo illustrates an extremely important and evidently very evanescent stage in the transferal of the veno-lymphatic sac to the definite lymphatic system as the jugular lymph sac. It has already been referred to in connection with the significance of the conditions shown by series 101 and 77 (figs. 50, 51 and 52) in which an anterior tap of evacuation was observed for the first time.

The main features furnished by the left side of this embryo are as follows:

1 The three primary divisions of the veno-lymphatic plexus of earlier stages (figs. 12 and 49) have fused with one another to form a common sac (fig. 57). As compared with series 77 (fig. 52), the relations of the spinal nerves and thyro-cervical artery to the resulting capacious sac clearly indicate the rôle played by the three primary divisions of the veno-lymphatic plexus in forming the sac.

2 The primary promontorial connections of earlier stages, which exist between the venous system and the plexus (Taps *B* and *C*, figs. 51 and 52) at the jugulo-subclavian and common jugular angles, have been completely lost as far as can be determined by the most careful observations. The jugular and subclavian approaches, however, form prominent processes of the sac, and terminate blindly near the point where they formerly communicated with the lateral surface of the promontory (fig. 51, 11

mm. embryo). In other words, the jugular and subclavian approaches retain exactly the same relations to the promontory, the cephalic vein and the thyro-cervical artery in the present case as they do in the 11 mm. embryo (figs. 51 and 52) where they communicate with the systemic veins.

The significance of the jugular and subclavian approaches, as well as their relation to the early veno-lymphatic plexus, should be constantly kept in mind in reading the following pages, since it is through them that the permanent connections between the lymphatic and venous systems are subsequently established.

3 Anteriorly, the veno-lymphatic sac opens by a broad orifice into the straight segment of the precardinal near the level of the cephalic arch, and is also connected with the same by a smaller channel slightly caudal to this point. This wide-open communication between the veno-lymphatic sac and the precardinal vein constitutes the *anterior tap of evacuation* which on the left side of this embryo is much more extensive in character than in the 10 and 11 mm. embryos (figs. 50, 51 and 52. This extensive entrance into the vein, which now serves as an outlet for the blood contents of the sac, is connected with the area of the precardinal which formerly received the dorsal tributaries **1, 2** and **3,** and its mode of origin in this case was probably the same as that of a similarly situated element which was described in connection with series 19 (9 mm., fig. 42).

The genesis of this anterior tap of evacuation can be further traced from the preceding 10 and 11 mm. stages, by comparing figs. 47, 48, 49, 50, 51 and 52 with fig. 57. A comparison of figs. 47, 48 and 49 with fig. 57 will show that the area of the tap of evacuation in the latter corresponds to the area of the veno-lymphatic derivatives of tributaries **1, 2** and **3,** as seen in the former, before confluence of the individual elements to form the conplete sac has occured.

In fig 50 (series 101, 10 mm., right side) the anterior tap of evacuation is small but distinct, the dorsal plexus arching to its precardinal junction over the combined trunks of the first and second spinal nerves. Cephalad of this point the junction of the arched and straight segment of the precardinal carries a dilated

and fenestrated appendage (fig. 50, **b**) which is also seen in series 77 (fig. 51, **b**). From the position of this element **b**, it seems probable that its junction with the small anterior tap of evacuation will establish the wide portal of entry, which in series 78 (fig 57) forms the main anterior connection of the sac with the veins. In this case it appears likely that the small caudal connection in series 78 (fig. 57) represents the original anterior tap as seen on the right side of series 101 (fig. 50), and on both sides of series 77 (figs. 50, 51, and 52). Finally, on the left side of series 101 (fig. 49), a stage is shown, just prior to the establishment of the anterior tap of evacuation, in which the dorsal veno-lymphatic arch has not yet united with the anterior precardinal veno-lymphatic elements to form the anterior tap of evacuation.

4. The blood contents of the earlier veno-lymphatic plexus have for the most part, been completely evacuated on the left side of this 12 mm. embryo (fig. 57), evidently through the large anterior venous connection just described. Here and there in the caudal portion of the sac small outlying pockets still contain a few blood cells, but as a whole the former veno-lymphatic plexus represents on this side an enormously expanded empty sac, dedetached at all points from the venous system except at the very large and the small anterior channels described. A comparison of the left (fig. 57) with the right side of this embryo (not figured) is most instructive. *On the right side the sac is completely closed, contains no blood corpuscles and, as far as we can determine, does not communicate with the veins at a single point.* Since the promontorial connections of the sac in both of the embryos which immediately precede this stage appear to be too insignificant to permit of the passage of a large amount of blood, we can only infer that on the right side, as on the left, an anterior tap of evacuation was at one time present, and that it served as the main exit for the blood contents of the veno-lymphatic sac. Figs. 53 and 54 (series 78, slide 3, sections 34 and 40) show the appearance of the anterior tap of evacuation and of the connected portion of the sac in transverse section on the left side, and, on the right side, the complete separation of the jugular sac from the veins. Figs. 55 and 56 show the caudal portion of the lymph sac (slide 4, sections 33 and 35).

The dorsal and ventral portions of the sac are clearly differentiated on the left side in Fig. 55. The multilocular character of the empty sac and the incomplete septa and partitions in the interior are well shown in both figures.

Secondary features of significance illustrated by the left side of this embryo are as follows:

1. The thyro-cervical artery on reaching the level of the promontory bends laterad on to the lateral surface of the promontory in the interval which separates the jugular from the subclavian approach. On reaching the lateral surface of the promontory the thyro-cervical artery gives off a branch which runs dorsally along the lateral surface of the veno-lymphatic sac. This latter branch is accompanied by a branch of the cephalic vein and in later stages both this vein and the artery which accompanies it may become embedded in the jugular lymph sac.

2. The primitive ulnar vein has given up its anterior connection with the promontory and the drainage of the anterior limb is now assumed by the definite subclavian vein. The subclavian vein opens into the promontory slightly caudal to the point where the latter formerly received the primitive ulnar vein. (Compare fig. 51, in which the subclavian connection is established). Before the primitive ulnar vein gives up its anterior connection with the promontory, the sixth spinal nerve (*SP.N.VI*) arches under or ventral to the primitive ulnar vein and primitive ulnar veno-lymphatic (figs. 45, 47 and 48). With the loss of its promontorial connection, however, this relation is alone maintained to the primitive ulnar veno-lymphatic (fig. 57). *Cf.* figs, 13, 14, 15 and 16.

3. The primitive ulnar veno-lymphatic forms a well-defined caudal prolongation of the veno-lymphatic sac and can be traced to the base of the anterior limb.

4. The marked convexity of the cephalic arch has been reduced with the descent of the heart and the resulting elongation of the straight segment of the precardinal. The promontory no longer forms a prominent swelling at the cardinal-Cuvierian junction, and has been reduced by the caudal descent of the heart.

5. The relations of the spinal nerves to the veno-lymphatic sac have already been referred to and need no further explanation.

6. A new element has been added to the veno-lymphatic sac which Lewis has described in connection with the lymph sac of the rabbit under the name of the subcutaneous duct. It consists of an appendage of the dorsal arch of the veno-lymphatic sac, which is connected with the lateral surface of the same at two points. This appendage increases in size from now on, and, as it is constant in its appearance, is worthy of consideration. As we have not followed its origin closely, however, we are unable to speak authoritatively concerning it.

Series 474, Harvard Embryological Collection, slides C, D, E, and F.
10.7^{mm} Embryo
Reconstruction of left side,
Lateral aspect fig. 58 and
Reconstruction of right side,
Lateral aspect, fig. 59

We owe the opportunity of studying and reconstructing this very important and interesting embryo to the courtesy of Prof. C. S. Minot of Harvard University. The length of the embryo is given as 10.7 mm., but in the development of its jugular lymph sac, it corresponds to specimens of our collections, which measure considerably more (from 11 to 12.5 mm.). This discrepancy may, however, be due to different technique in taking the total length measure, as to unusual curvature of the embryo.

Serially, from the developmental aspect, it follows the stage previously described (series 78, 12 mm.), and is an excellent example of the apparent complete bilateral temporary separation of the voided veno-lymphatic sac, now the closed and empty jugular lymph sac, from the permanent veins. The extremely doubtful connection of the sac with the venous system found by us near the jugulo-subclavian angle is confined to a single section on the right side (slide E, section 194), and to two sections on the left side (slide E, sections 197-198). Even if this connection exists, it differs entirely from both the earlier veno-lymphatic and the later definite lymphatico-venous taps. As previously stated (p. 194), if the empty sac separates temporarily from the venous system, and subsequently rejoins the same by establishing

secondary connections, it is to be expected that certain embryos will be fixed at periods in which, on the one hand, the temporary detachment has been nearly, but not quite completely attained, and, on the other, in which the secondary connections are just beginning to be established. In either case the resulting histological picture will yield these so-called "doubtful" connections. The evidence that the jugular lymph sac becomes temporarily separated from the veins is based upon the following observations:

1. We have been unable to find any connection between sac and vein on the right side of embryo 78 (12 mm.).

2. There is no connection on the left side of this embryo series 78 at either of the typical points of final lymphatico-venous entry, viz., the common jugular confluence and the jugulo-subclavian angle.

3. No connection between sac and veins exists at any point in a 11 mm. embryo (series 27), in which the jugular lymph sac is fully established.

4. No connection is found in the 13 mm. embryos.

5. Absence of the typical communications in the Harvard series 474 (10.7 mm), although the minute doubtful connections above described may be present.

The definite and permanent connections of the jugular lymph sac and the veins do not appear prior to the stage between 14 and 15 mm., from which stage on they are usually constant.

Reconstruction of left side, lateral aspect (slides C, D, E, and F) fig. 58.

The jugular lymph sac contains no blood and is composed of clearly defined confluent elements, (fig. 58), as follows:

1. The part derived from the cephalic division of the ventral veno-lymphatic plexus (*I*).

2. The part derived from the caudal division of the ventral veno-lymphatic plexus (*II*) terminating *in a blind prolongation* (jugular approach), which is directed toward, and nearly reaches, the angle of confluence of the internal jugular and the combined trunk of the external jugular and cephalic veins (common jugular angle).

3. The entire dorsal arch is derived from the dorsal veno-lymphatic plexus and the veno-lymphatic components of the precardinal tributaries, and terminates caudally in a pointed extremity (subclavian approach), which is directed toward the jugulo-subclavian angle, near which it forms a doubtful connection with the venous system (slide E, sections 197-198).

4. The primitive ulnar lymphatic, derived from the primitive ulnar veno-lymphatic. This structure now contains no blood cells, is distinctly lymphatic in structure, and forms a blind appendage to the caudal end of the jugular sac, arching dorso-ventrad over the brachial plexus, the sixth spinal nerve of which is represented in the figure *SP. N. VI.*

These components of the jugular lymph sac can readily be homologized with their equivalents in series 102, (fig. 46), series 101 (fig. 49), series 77(fig. 51) and series 78 (fig. 57).

The relations of the spinal nerves to the jugular lymph sac in the 10.7 mm. embryo remain fundamentally the same as in the later veno-lymphatic and pre-lymphatic stages and may be compared with those in figs. 49, 50, 52 and 57.

The external jugular terminal which opens singly into the promontory in series 102, (fig. 46), has united with the base of the cephlic vein, forming the common jugulo-cephalic trunk so characteristic of later stages.

In this, as well as in all subsequent stages, the promontory is no longer recognizable as an expanded and dilated portion of the main venous channel, but its former position is easily determined by the relations of the thyro-cervical artery and by the presence of a series of dorsal somatic tributaries which open into the jugular vein near the caudal end of the jugular lymph sac. These tributaries have been fully described in connection with the preceding stages (see **4S, 5S, 6S, 7S** and **8S**, fig. 45).

Reconstruction of Right side, Lateral Aspects (slides C, D, and E), Fig. 59.

The right side of this embryo presents identically the same conditions as the left, including the presence of a doubtful lymphatico-venous connection near the jugulo-subclavian angle (slide *E* section 194), with the exception that the cephalic and caudal divis-

ions of the ventral arch of the lymph sac have not united to form the ventral boundary of the large foramen through which the first and second (*SP.N.I* and *II*) and third and fourth (*SP.N.III* and *IV*) spinal nerves pass on the left side (fig. 58). The fifth spinal nerve (*SP.N.V*), as on the left side, and as in all of the preceding stages, penetrates the lymph sac.

LYMPHATIC STAGE OR STAGE OF THE DEFINITELY ORGANIZED JUGULAR LYMPH SAC.

Series 37, 14^{mm} Embryo
Series 15, 16^{mm} Embryo
Series 36, 17^{mm} Embryo
Series 88, 18^{mm} Embryo
Series 22, 25^{mm} Embryo

As mentioned above the jugular lymph sac consists of a single sac, containing no blood or only isolated corpuscles, which, after its apparent temporary separation from the veins in the preceding pre-lymphatic stage, now secondarily rejoins the venous system through its jugular and subclavian approaches at the common jugular confluence, or at the jugulo-subclavian angle, or at both points, depending upon the definite type of adult lymphatico-venous connection to be established in the individual instances. (See p. 188). With the assumption of this definite lymphatic stage the organization of the jugular sac is completed. The further development involves merely some topographical changes in relation to the spinal nerves and the establishment of secondary connections with the independently formed systemic lymphatics.

Series 37, 14^{mm} Embryo
Reconstruction of left side,
Dorsal aspect, fig. 60 and
Ventral aspect, fig. 61

Only the caudal end of the lymph sac is shown in this reconstruction.

The jugular lymph sac is much enlarged in this embryo, and the subcutaneous duct is still a prominent tributary of the sac.

The internal jugular vein has relatively decreased in size, while the external jugular and cephalic veins have correspondingly enlarged. The two divisions of the lymph sac (dorsal and ventral) have become closely amalgamated in the caudal portion and the united sac is still perforated some distance from its dorsal margin by the fifth cervical nerve (*S.P.N.V.*). The sac terminates caudally in four prolongations:

1. The jugular approach (fig. 61) terminates in a pointed extremity, which enters, as previously described, in a wedged-shaped invagination, with valve formation, the dorsal aspect of the angle of confluence of the internal jugular (precardinal) and the combined trunk of the external jugular and cephalic veins. This forms the typical *secondary* and final lymphatico-venous tap at the common jugular confluence. (Compare with figs. 1, 2 and 3 of adult).

2. The subclavian approach (fig. 61), is a much elongated process of the ventro-caudal end of the jugular sac, which extends caudad, ventral to and somewhat beyond the jugulo-subclavian junction, where it ends in a pointed blind extremity. A doubtful point of connection exists with the vein near the jugulo-subclavian junction. The subclavian approach is potentially in position to establish a typical secondary entrance into the dorsal aspect of the jugulo-subclavian angle. We have, therefore, probably here an instance in which, while the common jugular secondary lymphatico-venous tap is already established, the subclavian connection has not yet been made. It is, of course, on the other hand, possible that we are dealing with an individual which in the adult state would have presented only a single (common jugular) lymphatico-venous connection, and in which the secondary jugulo-subclavian tap was never formed. (Fig. 1, left side).

3. The third prolongation is formed by the primitive ulnar lymphatic as a blind process of the sac, arching caudad over the sixth spinal nerve (fig. 61, *S.P.N.VI.*), along the dorsal aspect of the subclavian vein.

4. The point at which in later stages the proximal end of the thoracic duct will connect with the jugular sac, is represented (fig. 60), by a short process which lies upon the dorsal surface

of the internal jugular vein, between the sympathetic nerve and the thyro-cervical artery. It extends caudad as far as the base of the first of the (promontorial) dorsal somatic tributaries (**4S** in figs. 34, 45 and 60), where it ends blindly. The connection of this process with the jugular sac lies dorsal to that of the jugular approach, the position of the latter, on the ventral surface of the vein, being represented by a plus sign (+) in fig. 60.

The thyro-cervical artery, a branch of the subclavian, runs cephalad along the dorsal-lateral surface of the jugular vein and on reaching the lymph sac turns abruptly laterad, ventral to the subclavian approach, and then divides into three branches one of which runs dorsad along the lateral surface of the jugular sac. The relations of the thyro-cervical artery to the jugular sac are represented in fig. 60. The branch which follows the lateral surface of the lymph sac and its accompanying vein are not represented, however. It will be observed that the relations of the thyro-cervical artery to the jugular lymph sac are exactly the same as in all of the preceding stages (figs. 49, 51, 52 and 57).

The external jugular vein is a vein of large size in this embryo (fig. 61), having practically assumed its adult condition. It usually functions as the main drainage canal of the head and neck in the adult although cases are sometimes met with in which the external and internal (precardinal) jugulars are subequal in size.

Series 15, 16^{mm} Embryo
Reconstruction of left side,
Dorso-lateral aspect, fig. 62

The jugular lymph sac now appears relatively reduced and shortened in its cephalo-caudal dimension. It ends blindly in front in a bluntly pointed extremity, and receives the subcutaneous duct on its lateral surface.

A number of features characterize this embryo, which show an advance in lymphatic development over that of the preceding stage (14 mm.).

1. The jugular approach, not shown in the dorso-lateral view, communicates with the venous system at the angle of confluence of the internal jugular and the combined trunk of the external

jugular and cephalic veins (common jugular tap). This lymphatico-venous connection in no way resembles those of the early veno-lymphatic, nor the doubtful ones in the lymphatic stages, but is established by a wedge shaped process of the lymph sac which is deeply invaginated into the angle of confluence of two veins and opens into the vascular lumen by a narrow slit-like aperture bounded by a two-lipped valve. This type of valve, also characteristic of the adult, is illustrated by figs. 4, 5, 6 and 7, which are photomicrographs of transverse sections through the region of the valve in the embryo under consideration.

2. The subclavian approach also communicates with the venous system at the jugulo–subclavian junction by a narrow slit-like aperture (jugulo-subclavian tap).

In view of the conditions observed in the preceding stages we are bound to infer that these two taps have been secondarily formed.

By comparing fig. 62 with fig. 3, it will be seen that the adult condition has been reached.

3. The separate anlages of the thoracic duct, formed independently of the jugular lymph sac, have now united with each other, and with the dorsal process of the lymph sac above described. The thoracic duct, therefore, for the first time, now forms a continuous vessel between the posterior thoracic region and the jugular lymph sac into which it opens dorsal to, and slightly in front of the tap at the common jugular confluence. In the portion of the embryo figured the thoracic duct crosses the dorsal surface of the subclavian artery, passes ventral to the sympathetic nerve, and from this point on to its entrance into the jugular sac it lies between the sympathetic nerve and the thyro-cervical artery. Along this course it follows the dorsal surface of the left innominate and common jugular veins.

4. The primitive ulnar lymphatic has lost its connection in this embryo with the jugular sac, as may be seen by the relations of the sixth spinal nerve (*SP.N. VI*, fig. 62). It may be stated in general that the primitive ulnar lymphatic retains its connection with the jugular sac through the 14 mm. stage, after which this connection appears to be lost. This change, therefore,

approximately coincides with, or follows very shortly upon, the establishment of the subclavian vein as the chief channel of venous return from the anterior limb and the resulting abolishment of the primitive ulnar vein.

The further fate of the primitive ulnar lymphatic is involved in the establishment of the definite systemic lymphatic channels of the anterior extremity, and is hence not considered at this time.

5. The relations of the thyro-cervical artery to the subclavian approach and to the common trunk formed by the external jugular and cephalic veins, is well illustrated by the series of photomicrographs taken through this region, and should be compared with the reconstruction (fig. 62). Beginning caudad, figs. 7, 6, and 5 represent the thyro-cervical artery situated dorsal to the common trunk of the external jugular and cephalic veins and medial to the subclavian approach. Fig. 4, on the other hand, represents a transverse section taken through the thyro-cervical artery as it bends laterad, ventral to the subclavian approach and dorsal to the jugulo-cephalic trunk. This last section lies slightly caudal to the point where the thyro-cervical artery divides into three branches; one ventrally directed branch; one which follows the cephalic vein, and another which runs dorsally along the lateral surface of the jugular sac and is accompanied by a branch of the cephalic vein (fig. 62). The latter branch as well as the vein, as mentioned above, are at times embedded in the lymph sac (see fig. 64, series 88).

6. The first, second, third and fourth (fig. 62, *SP.N. I, II, III,* and *IV*) spinal nerves no longer pass through a foramen in the lymph sac as in fig. 57, but now pass between the sac and the internal jugular (precardinal) vein. The fifth (*SP.N.V*) spinal nerve still penetrates the jugular sac while the sixth (*SP.N. VI*) lies upon the lateral surface of the subclavian approach, and, as stated above, is now in no way related to the primitive ulnar lymphatic.

The present relations of the first four spinal nerves to the jugular sac are explained by comparing fig. 17 with the preceding fig. 16. In course of further development, as illustrated in the present instance, the large lymphatic foramen through which

the nerves pass, is broken by reduction of its anterior extremity. The lymph sac now is continued cephalad into two processes, a smaller ventral and larger dorsal. The former, which accompanies the internal jugular vein, becomes further reduced and recedes caudad, thus freeing the nerves and allowing them to pass between the internal jugular vein and the larger dorsal process which remains as the main component of the lymph sac in this stage.

Series 36, 17^{mm} Embryo
Reconstruction of left side,
Dorso-lateral aspect, fig. 63

The general composition of the caudal end of the jugular lymph sac, which is alone represented in the figure, corresponds quite closely to the 16 mm. stage.

A lymphatico-venous connection, as in the 16 mm. embryo, has been established on the dorsal aspect of the common jugular confluence (common jugular tap) by the jugular approach. This tap cannot be seen from the dorsal view of the reconstruction but its relative position is indicated by a plus sign (+) in the figure.

The jugular sac is prolonged caudad into a subclavian approach which now bifurcates near the jugulo-subclavian junction into a dorsal and ventral division. The ventral division constitutes the caudal extension of the original subclavian approach of the preceding stages and in the present embryo communicates ventrally with the veins at the jugulo-subclavian junction (jugulo-subclavian tap). The dorsal division, not present in the 16 mm. embryo, extends caudad for a short distance on the dorsal aspect of the jugulo-subclavian angle where it ends blindly. It is now in a position, however, to establish the secondary dorsal lymphatico-venous connection at this point which is frequently met with in the adult.

The thoracic duct is represented by a more complex system of vessels in this embryo than in the 16 mm. stage. A number of vessels from the thoracic region converge to form a single trunk which unites with the jugular sac dorsal to and slightly in front of the common jugular tap. The relations of the thoracic duct

to the subclavian, thyro-cervical arteries and to the sympathetic nerve remain the same as described above for the 16 mm. embryo.

Two small lymphatic vessels lying along the internal jugular vein open into the medial aspect of the jugular sac just in front of the entrance into the latter of the thoracic duct. These vessels represent the approach of the sac to union with the lymphatic vessels which have begun to form along the course of the internal jugular vein.

The fifth spinal nerve (*SP.N.V.*) still penetrates the jugular sac and the relations of the thyro-cervical artery and its branches to the jugular lymph sac and systemic veins are the same as in the 16 mm. embryo (fig. 62).

Series 88, 18^{mm} Embryo

Reconstruction of the jugular lymph sac of the right and left sides
Ventral View, fig. 64 and
Lateral view of the left side, fig. 66

This reconstruction is especially valuable in showing the symmetrical degree of development obtained by the jugular lymph sac on opposite sides of the same embryo, as well as the fact that up to a certain stage of development both sacs are ontogenetically of equal importance (fig. 64).

In the 18 mm. embryo the arteries and main systemic veins have practically reached the adult condition. The external and internal jugulars are more elongated than in the preceding stage as the result of the continued descent of the heart. The external jugular is a vein of large size, although its caliber does not yet exceed that of the internal jugular. The cephalic vein is also of large size and, after joining the external jugular to form the common jugulo-cephalic trunk, the latter, which has become somewhat elongated, joins the internal jugular at the common jugular angle. The resulting common trunk formed by the confluence of the internal jugular and jugulo-cephalic trunk, as far caudad as its confluence with the subclavian, now constitutes the common jugular vein (see fig. 1 of adult).

Beginning in the 17 mm. embryo, slightly caudal to the level of the subclavian vein and dorsal to the thymus glands, the two

precaval veins (precardinal) anastomose with each other across the median line and thereby establish the anlage of the definite right and left innominate (brachio-cephalic) veins. In the 18 mm. embryo this anastomosus is completed and the blood from the left jugular and left subclavian districts is now, for the most part, directed into the right precava. As the result of this transfer the right precava in the 18 mm. embryo has become much enlarged and the left correspondingly reduced.

In addition to the innominate anastomosis, mentioned above, another anastomosis occurs in this region ventral to the thymus gland. This makes its appearance in the 15 and 16 mm. embryos, and before the definite innominate anastomosis has been established; but with an increase in size of the latter, this anastomosis soon disappears. In the 18 mm. embryo, however, the thymus is still crossed ventrally by a net-work of vessels which represents the remains of this sub-thymic anastomosis.

The transferal of the blood current from the left jugular and left subclavian veins to the right precava is illustrated by figs. 13, 14, 15, 16 and 17 to which the reader is referred. An examination of these figures will make plain the conditions of the venous system in this (figs. 64 and 65), and in the embryo next to be considered (25 mm., fig. 66).

Lymphatic development has reached an advanced stage in the 18 mm. embryo. The capacious jugular sac of each side has established in this instance the typical taps at the common jugular and jugulo-subclavian angles, respectively. (Cf. fig. 3 of adult). The left jugular sac also receives the large thoracic duct on its dorsal aspect. The lateral view of the left side of the reconstruction (fig. 65), shows this connection.

The jugular sacs present further evidence of the reduction of the ventral portion, the main sac being formed by the dorsal part of the earlier lymphatic ring surrounding the first four spinal nerves. As a result of this change, with the exception of the fifth nerve (fig. 64, *SP.N.V*), which still penetrates through the sac, the remaining four anterior nerves pass between the sac and the internal jugular vein and the lymphatics which accompany that vessel.

The branch of the thyro-cervical artery which extends dorsad

along the lateral surface of the jugular sac in earlier stages (fig. 62) is embedded in the lymph sac in this 18 mm. embryo (fig. 64) with an accompanying branch of the cephalic vein.

Lymphatic formation, independent of the jugular lymph sac, is in full swing, and some of these independent lymphatics have made secondary connections with the jugular sacs. Closed lymphatic channels or sacs stud the course of the jugular, cephalic and subclavian veins, and an especially prominent lymphatic follows the course of the diminishing left precava (fig. 65).

Series 22, 25^{mm} Embryo
Reconstruction of left side,
Ventral aspect, fig. 66

Only the caudal end of the lymph sac and its related veins are shown in the figure.

As far as the jugular lymph sac is concerned, the final approach to the adult condition is achieved in the 25 mm. embryo. There is a further reduction in the size of the internal and a corresponding enlargement of the external jugular vein. The common vessel (jugulo-cephalic trunk) formed by the confluence of the external jugular and cephalic veins has, with further cardiac descent, become much elongated and the common jugular angle lies relatively further caudad than in the 18 mm. embryo. This descent has drawn out the lymph sac of the preceding stage into an elongated channel which lies along the veins and taps them in this case also at the two typical points.

The dorsal and ventral divisions of the subclavian approach, described above in connection with the 17 mm. embryo (fig. 63), now anastomose with each other just caudal to the jugulo-subclavian confluence, where they become continuous with a lymph channel which follows the left precava. This latter lymphatic was described in connection with the 18 mm. embryo (left prevacal lymphatic, fig. 65), where a connection between it and the jugular sac does not yet exist.

Numerous independent lymphatic structures are found along the course of the large veins. Among these may be mentioned a large complex lymphatic sac which envelops the subclavian vein,

and which does not yet join the jugular sac, as far as we can determine. This lymphatic complex increases in its dimensions from before backward.

An elongated and fenestrated lymphatic vessel, not yet joined to the subclavian approach, lies ventral to the left precava and the anlage of the left innominate vein, while another isolated lymphatic is found at the jugulo-cephalic junction. None of these lymphatic structures communicate with the veins.

The lymphatic organization of this embryo foreshadows the conditions in the adult.

The innominate cross anastomosis, the establishment of the right and left innominate veins and the reduced size of the left precava, are well shown in the 25 mm. embryo (fig 66).

IV. GENERAL CONSIDERATIONS AND CONCLUSIONS

In the preceding pages the writers have presented a detailed account of the development of the jugular lymph sacs in the domestic cat (*Felis domestica*).

The primary principles underlying the development of the jugular lymph sac are: (1) the development of a secondary channel parallel to the embryonic precardinal and the Cuvierian end of the postcardinal; (2) the association with this secondary channel of a certain number of dorsal precardinal tributaries, and (3) the separation of these two sets of venous elements, which we have termed 'Veno-lymphatics', from the main venous channels, and their subsequent conversion into the definite jugular lymph sacs by a process of growth and fusion.

For a complete and short résumé of the development of the jugular lymph sac, illustrated by diagrams (figs. 8 to 21), the reader is referred to the topic in the preceding pages, entitled 'Analysis of Developmental Stages in the Formation of the Jugular Lymph Sacs' (page 202). A short résumé, although less complete than the one referred to, was published in the Anatomical Record, Vol. II, 1908, to which the reader is also referred.

The writers wish to make it perfectly clear that the present communication deals solely with the anatomy and development of the JUGULAR LYMPH SACS of the cat, and *does not* touch on the question of the origin of the independently formed systemic lymphatic vessels which unite with the jugular sacs.

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We take pleasure in expressing our thanks and gratitude to our friend Mr. M. Petersen for his untiring efforts in our behalf, as well as our appreciation of the manner in which he has reproduced our complicated reconstructions for publication.

EXPLANATION OF FIGURES

Figs. 1, 2, and 3. Dissections of the lymphatics in the neck region of the adult cat (*Felis domestica*), showing the three normal forms of lymphatico-venous communication which may occur on each side of the body, namely, at the common jugular angle, at the jugulo-subclavian angle or at both of these points.

Figs. 4, 5, 6 and 7. Photomicrographs of transverse sections of a 16 mm. cat embryo (series 15), sections 233, 237, 239 and 241, respectively, taken at the level of the junction of the left jugulo-subclavian trunk and left internal jugular vein (common jugular confluence), illustrating the type of lymphatico-venous connection met with in advanced stages at the two typical points of lymphatico-venous entry.

Figs. 8 to 11. Composite diagrams illustrating the development of the jugular lymph sacs in the cat. Lateral views of left side.

Fig. 11 A. Dorsal view of fig. 11 in the region of the jugular promontory.

Figs. 12 to 17. Composite diagrams, continued, illustrating the development of the jugular lymph sacs in the cat. Lateral views of left side.

Fig. 18. Composite diagram (dorsal view) of the precardinal vein and promontory of the left side, illustrating the separation of the precardinal tributaries **1, 2, 3** and **4** into their *dorso-lateral* veno-lymphatic and *dorso-medial* somatic components; also the relation of the dorsal veno-lymphatic plexus to the promontory and to the promontorial somatic tributaries, **5S, 6S,** etc.

Fig. 19. Diagram of precardinal vein and promontory (dorsal view), illustrating a case in which the para-precardinal channel is alone involved in the formation of the caudal division of the ventral veno-lymphatic plexus.

Fig. 20. Diagram of left precardinal vein and promontory (dorsal view), illustrating the rôle played by the veno-lymphatic component of the precardinal tributary **4** (4VL) in forming the rounded appendage of the caudal division of the ventral veno-lymphatic plexus, sometimes met within the later stages.

Fig. 21. Diagram of left precardinal vein and promontory (dorsal view) illustrating a case in which the para-precardinal channel has given up its connection with the jugular promontory, leaving thereon a small conical teat-like process near the future common jugular junction.

ALL OF THE RECONSTRUCTIONS MENTIONED BELOW INCLUDE THE REGION OF THE PRECARDINAL VEIN AND ITS CONFLUENCE WITH THE POSTCARDINAL VEIN AND THE DUCT OF CUVIER.[21]

Fig. 22. Reconstruction of a 5 + mm. cat embryo (series 30), left side, lateral aspect.

Fig. 23. Reconstruction of a 5 + mm. cat embryo (series 30), right side, lateral aspect.

Fig. 24. Reconstruction of a 5 + mm. cat embryo (series 31), left side, lateral aspect. The postcardinal vein was not reconstructed in this embryo.

Fig. 25. Reconstruction of a 5 mm. cat embryo (series 134), left side, lateral aspect.

Fig. 26. Reconstruction of a 5 mm. cat embryo (series 47), right side, lateral aspect.

Fig. 27. Reconstruction of a 6.2 mm. cat embryo (series 109), left side, lateral aspect.

Fig. 28. Reconstruction of 6.2 mm. cat embryo (series 109), left side, medial aspect.

Fig. 29. Reconstruction of a 6.2 mm. cat embryo (series 109), right side, lateral aspect.

Fig. 30. Reconstruction of 6.2 mm. embryo (series 109), right side, medial aspect.

Fig. 31. Reconstruction of a 7 mm. cat embryo (series 2), right side, lateral aspect.

Fig. 32. Reconstruction of a 7 mm. cat embryo (series 2), left side, lateral aspect.

Fig. 33. Reconstruction of a 7 mm. cat embryo (series 138), left side, lateral aspect.

Fig. 34. Reconstruction of a 7 mm. embryo (series 138), left side, medial aspect.

Fig. 35. Reconstruction of a 7 mm. cat embryo (series 138), right side, lateral aspect.

Fig. 36. Reconstruction of 7 mm. embryo (series 138), right side, medial aspect.

Fig. 37. Diagram (dorsal view) of fig. 35.

Fig. 38. Reconstruction of a 7.25 mm. cat embryo (series 13), right side, lateral aspect.

Fig. 39. Reconstruction of a 7.25 mm. cat embryo (series 13), left side, lateral aspect.

Fig. 40. Reconstruction of a 9 mm. cat embryo (series 106), left side, lateral aspect.

Fig. 41. Reconstruction of a 9 mm. cat embryo (series 19), right side, lateral aspect.

[21] In a few cases in which the first four spinal nerves (*SP.N.I-IV*) were omitted in the reconstructions they have been added to the drawings of the same in order to show the relations which they bear to the developing jugular lymph sac. In all other respects the drawings of the reconstructions are accurate reproductions of the models which they represent.

Fig. 42. Reconstruction of a 9 mm. cat embryo (series 19), left side. lateral aspect.

Fig. 43. Photomicrograph of a transverse section of a 10 mm. cat embryo (series 114, slide 4, section 27), taken at a level just anterior to the jugular promontory and showing the relative position of the dorso-lateral veno-lymphatic and dorso-medial somatic elements with respect to each other and to the precardinal vein.

Fig. 44. Reconstruction of an 8.5 mm. cat embryo (series 102), left side, lateral aspect.

Fig. 45. Reconstruction of an 8.5 mm. cat embryo (series 102), left side, medial aspect.

Fig. 46. Reconstruction of an 8.5 mm. cat embryo (series 102), right side, lateral aspect.

Fig. 47. Reconstruction of a 10 mm. cat embryo (series 112), left side, lateral aspect.

Fig. 48. Reconstruction of a 10 mm. cat embryo (series 113), left side, lateral aspect.

Fig. 49. Reconstruction of a 10 mm. cat embryo (series 101), left side, lateral aspect. (The dotted lines represent the actual cephalic and caudal limits of the area reconstructed).

Fig. 50. Reconstruction of a 10 mm. cat embryo (series 101), right side, lateral aspect.

Fig. 51. Reconstruction of a 11 mm. cat embryo (series 77), left side, lateral aspect.

Fig. 52. Reconstruction of a 11 mm. cat embryo (series 77), right side, lateral aspect.

Figs. 53 and 54. Photomicrographs of transverse sections of a 12 mm. cat embyro (series 78) passing through the *anterior tap of evacuation* on the left side and showing the absence of this tap on the right side of the embryo (sections 34 and 40, respectively, of slide 3).

Figs. 55 and 56. Photomicrographs of transverse sections of a 12 mm. cat embryo (series 78, slide 4, sections 33 and 35), passing through the caudal end of the jugular lymph sac, anterior to the jugular promontory. The multilocular character of the jugular sac is clearly shown in fig. 56.

Fig. 57. Reconstruction of a 12 mm. cat embryo (series 78), lateral aspect, left side.

Fig. 58. Reconstruction of a 10.7 mm. cat embryo (Harvard Embryological Collection, series 474), left side, lateral aspect.

Fig. 59. Reconstruction of a 10.7 mm. cat embryo (Harvard Embryological Collection, series 474), right side, lateral aspect.

Fig. 60. Reconstruction of a 14 mm. cat embryo (series 37), left side, dorsal aspect.

Fig. 61. Reconstruction of a 14 mm. cat embryo (series 37), left side, ventral aspect.

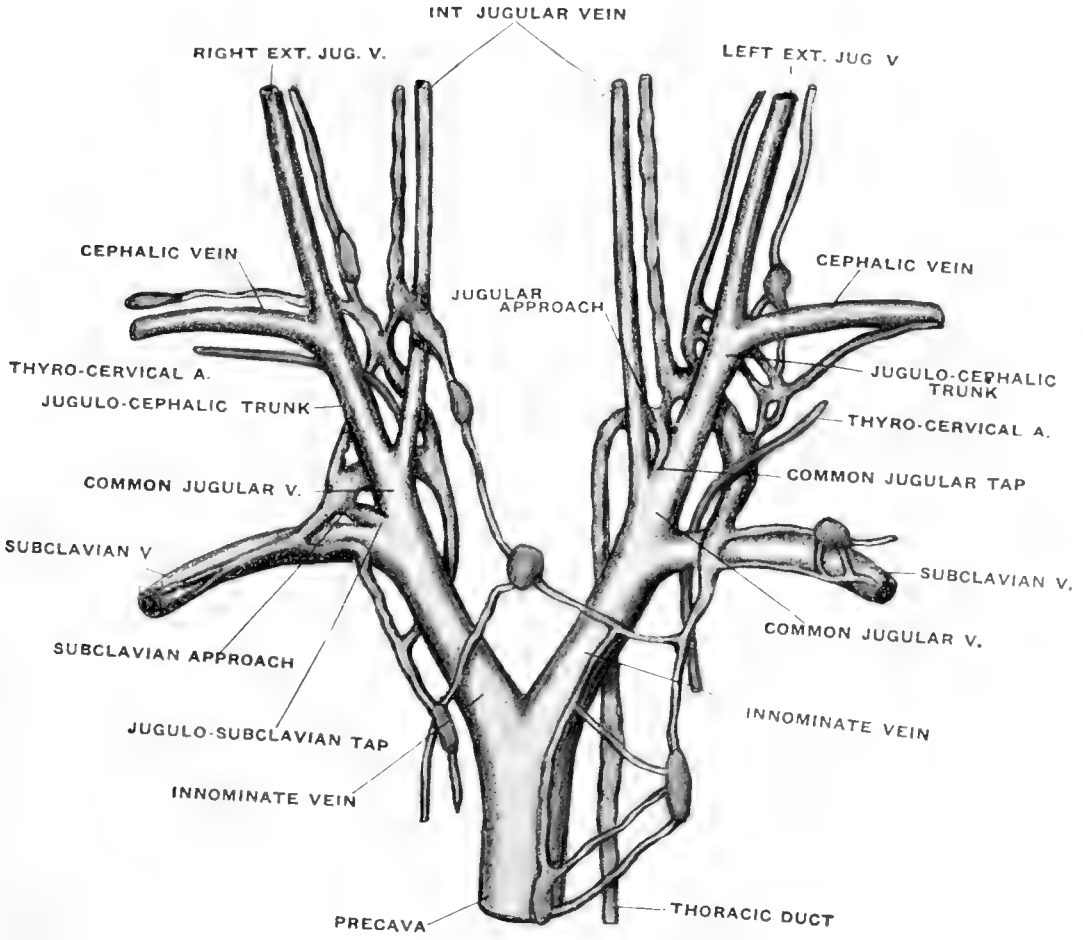
Fig. 62. Reconstruction of a 16 mm. cat embryo (series 15), left side, dorsolateral aspect.

Fig. 63. Reconstruction of a 17 mm. cat embryo (series 36), left side, dorsolateral aspect.

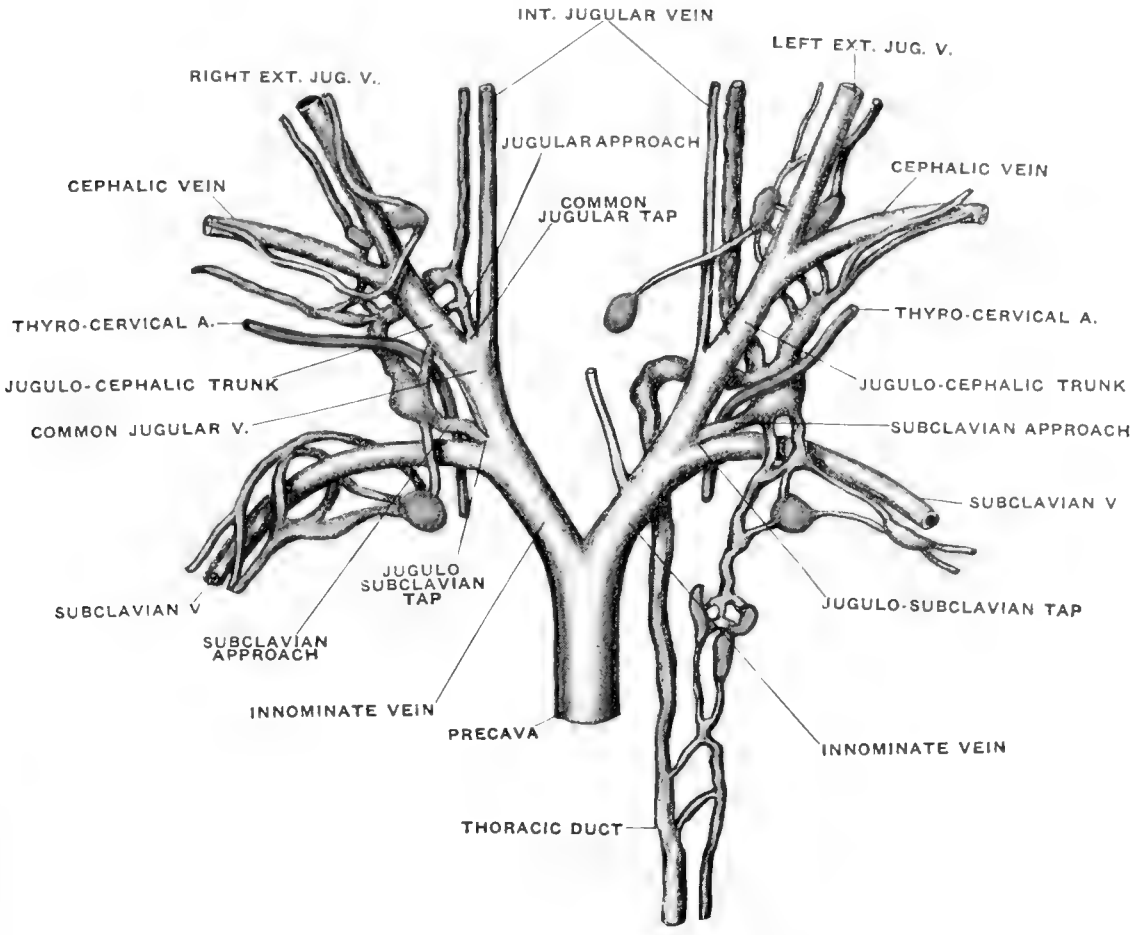
Fig. 64. Reconstruction of an 18 mm. cat embryo (series 88), both sides, ventral aspect.

Fig. 65. Reconstruction of an 18 mm. cat embryo (series 88), left side, lateral aspect.

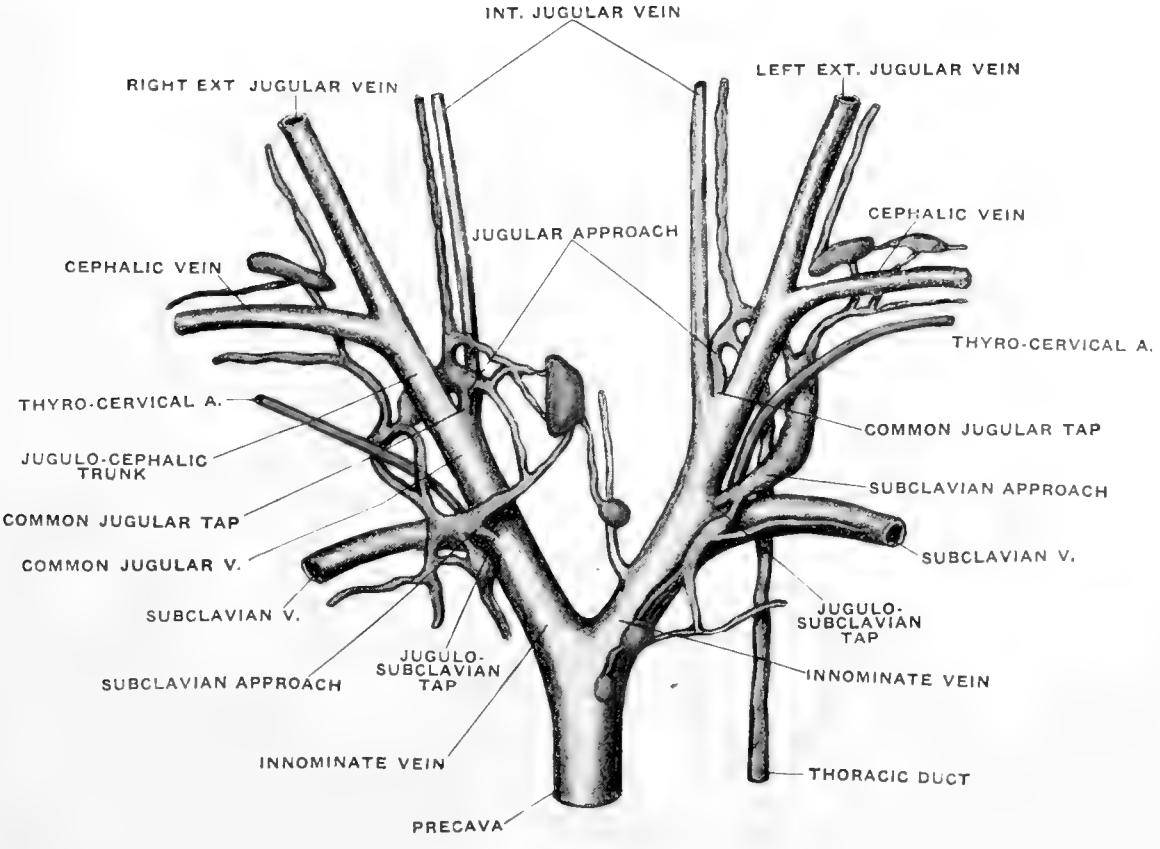
Fig. 66. Reconstruction of a 25 mm. cat embryo (series 22), left side, ventral aspect.


INT JUGULAR VEIN
RIGHT EXT. JUG. V.
LEFT EXT. JUG V
CEPHALIC VEIN
CEPHALIC VEIN
JUGULAR APPROACH
THYRO-CERVICAL A.
JUGULO-CEPHALIC TRUNK
JUGULO-CEPHALIC TRUNK
THYRO-CERVICAL A.
COMMON JUGULAR V.
COMMON JUGULAR TAP
SUBCLAVIAN V
SUBCLAVIAN V.
COMMON JUGULAR V.
SUBCLAVIAN APPROACH
INNOMINATE VEIN
JUGULO-SUBCLAVIAN TAP
INNOMINATE VEIN
PRECAVA
THORACIC DUCT

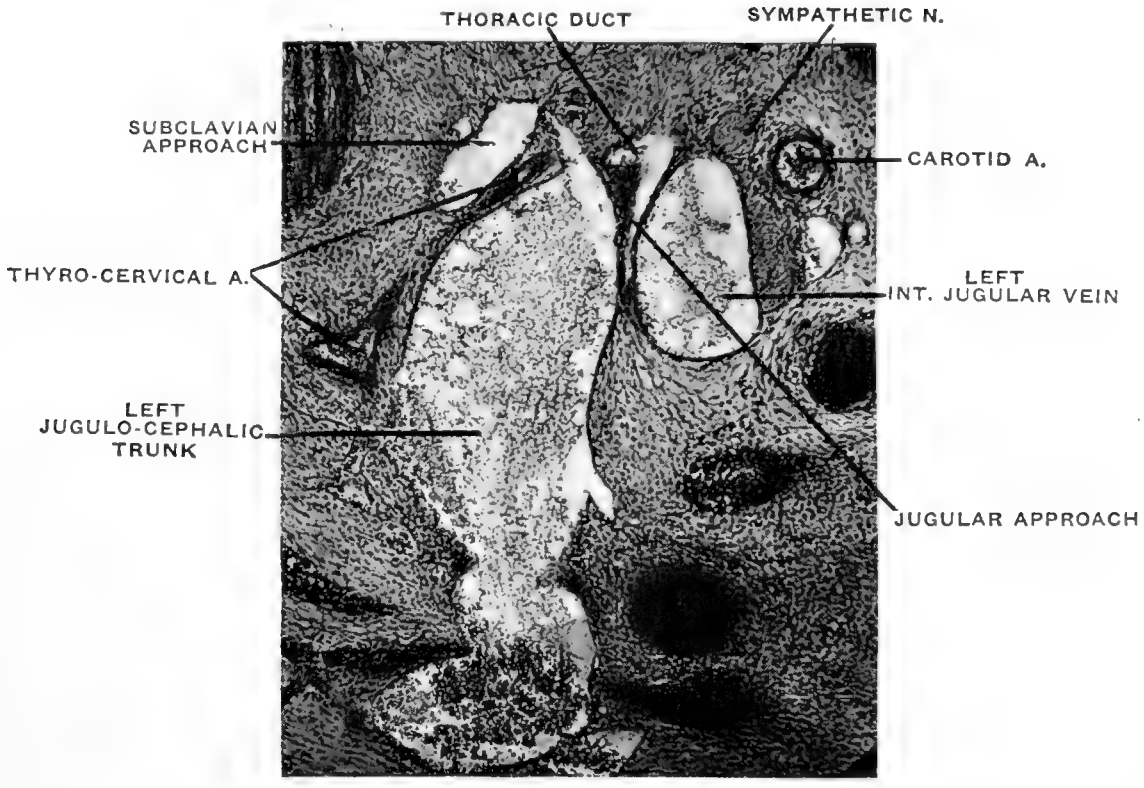
1


INT. JUGULAR VEIN
LEFT EXT. JUG. V.
RIGHT EXT. JUG. V.
JUGULAR APPROACH
CEPHALIC VEIN
CEPHALIC VEIN
COMMON JUGULAR TAP
THYRO-CERVICAL A.
THYRO-CERVICAL A.
JUGULO-CEPHALIC TRUNK
JUGULO-CEPHALIC TRUNK
COMMON JUGULAR V.
SUBCLAVIAN APPROACH
SUBCLAVIAN V
JUGULO SUBCLAVIAN TAP
SUBCLAVIAN V
JUGULO-SUBCLAVIAN TAP
SUBCLAVIAN APPROACH
INNOMINATE VEIN
PRECAVA
INNOMINATE VEIN
THORACIC DUCT

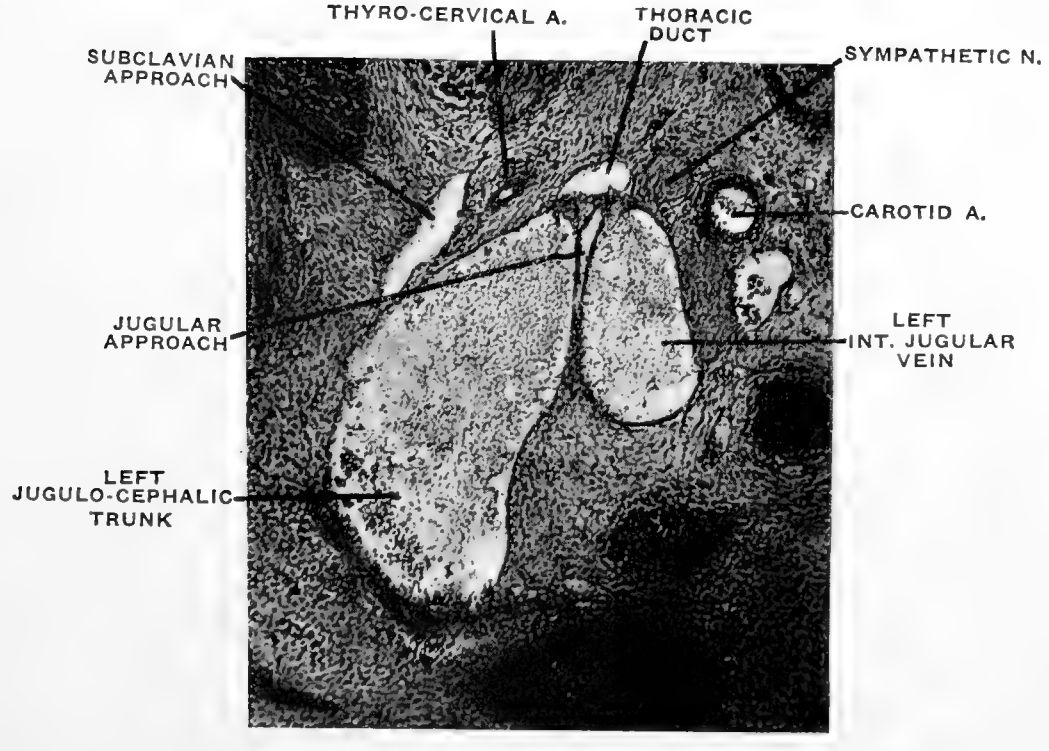
2


INT. JUGULAR VEIN
RIGHT EXT JUGULAR VEIN
LEFT EXT. JUGULAR VEIN
CEPHALIC VEIN
JUGULAR APPROACH
CEPHALIC VEIN
THYRO-CERVICAL A.
THYRO-CERVICAL A.
COMMON JUGULAR TAP
JUGULO-CEPHALIC TRUNK
SUBCLAVIAN APPROACH
COMMON JUGULAR TAP
SUBCLAVIAN V.
COMMON JUGULAR V.
JUGULO-SUBCLAVIAN TAP
SUBCLAVIAN V.
JUGULO-SUBCLAVIAN TAP
SUBCLAVIAN APPROACH
INNOMINATE VEIN
INNOMINATE VEIN
THORACIC DUCT
PRECAVA

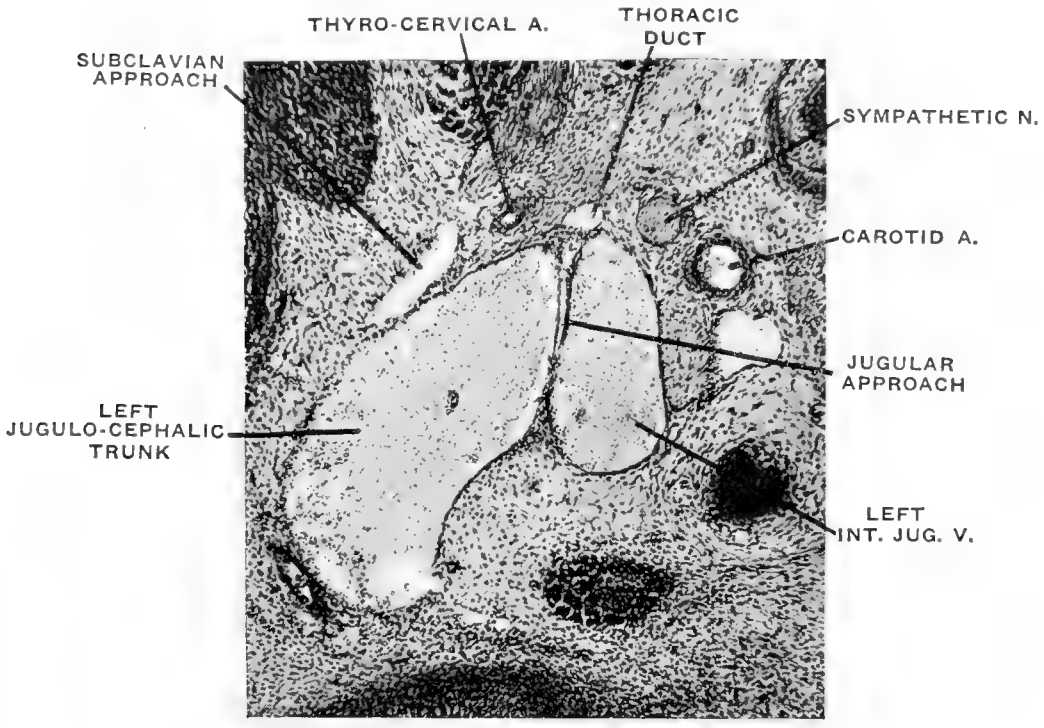
3


THORACIC DUCT
SYMPATHETIC N.
SUBCLAVIAN APPROACH
CAROTID A.
THYRO-CERVICAL A.
LEFT INT. JUGULAR VEIN
LEFT JUGULO-CEPHALIC TRUNK
JUGULAR APPROACH

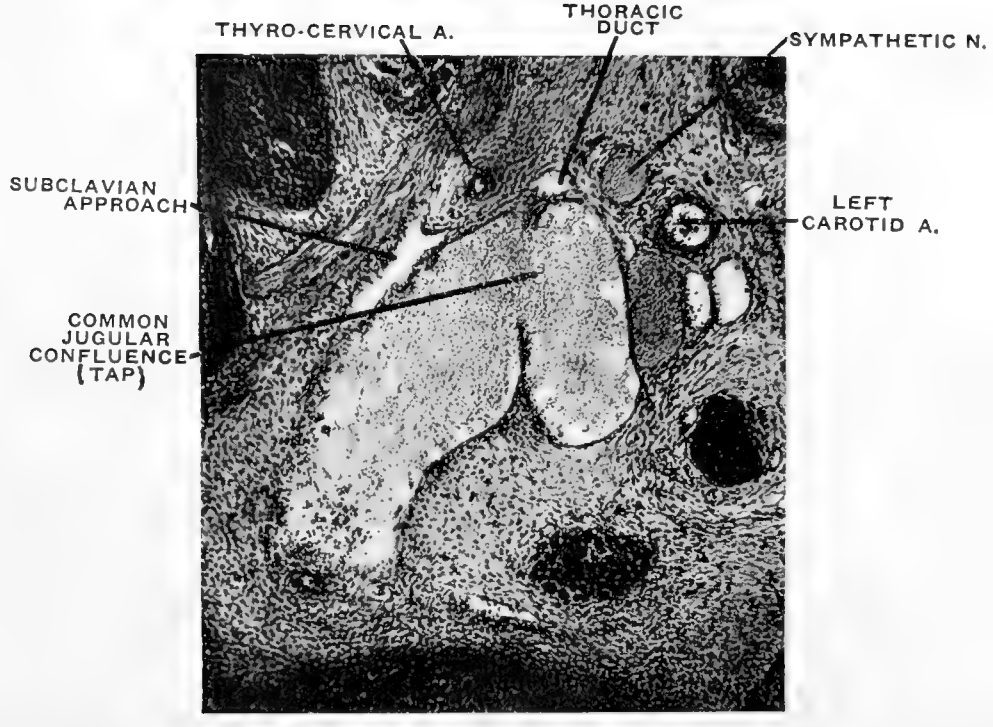
4


THYRO-CERVICAL A.
THORACIC DUCT
SUBCLAVIAN APPROACH
SYMPATHETIC N.
CAROTID A.
JUGULAR APPROACH
LEFT INT. JUGULAR VEIN
LEFT JUGULO-CEPHALIC TRUNK

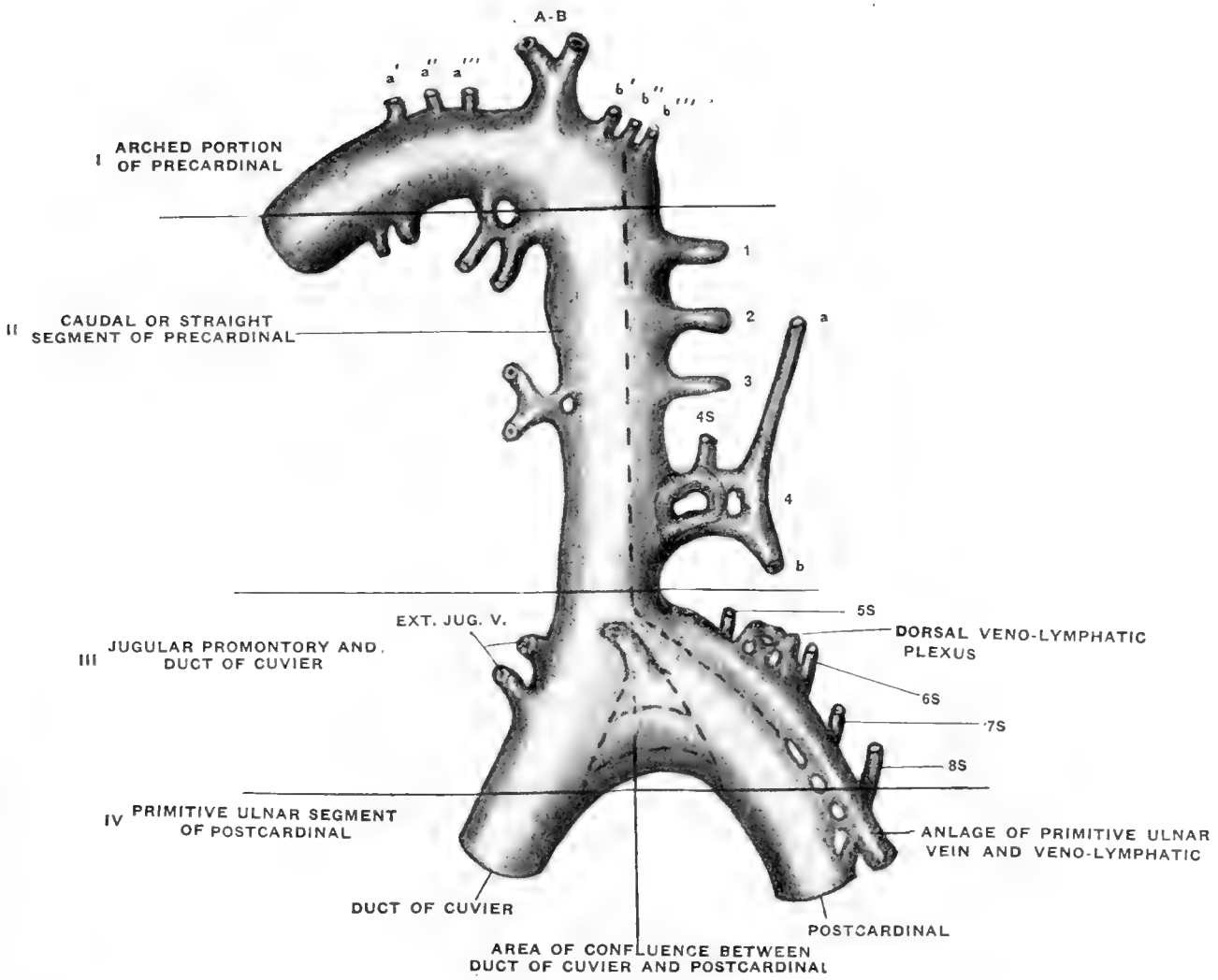
5

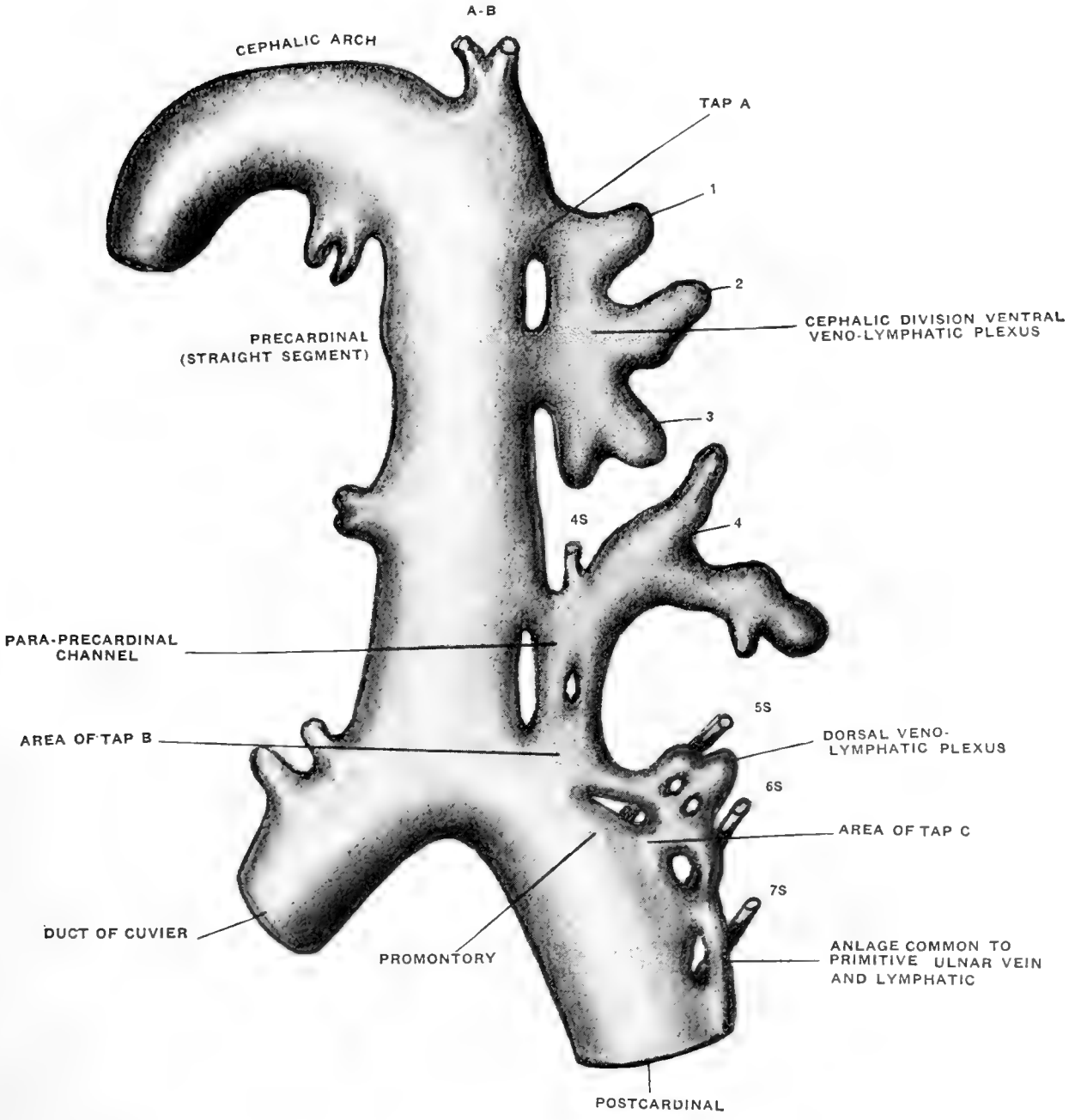

THYRO-CERVICAL A.
THORACIC DUCT
SUBCLAVIAN APPROACH
SYMPATHETIC N.
CAROTID A.
JUGULAR APPROACH
LEFT JUGULO-CEPHALIC TRUNK
LEFT INT. JUG. V.

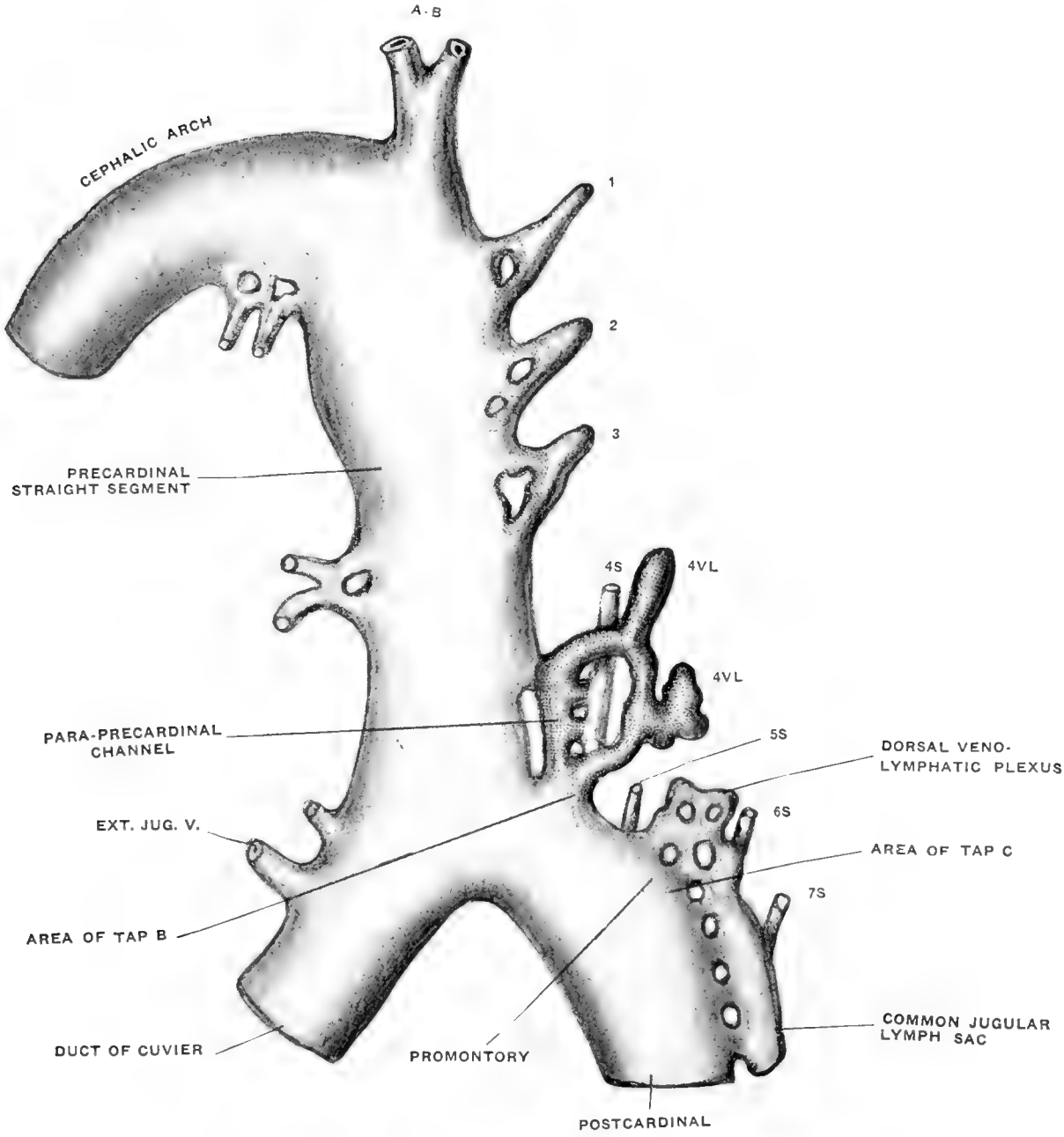
6

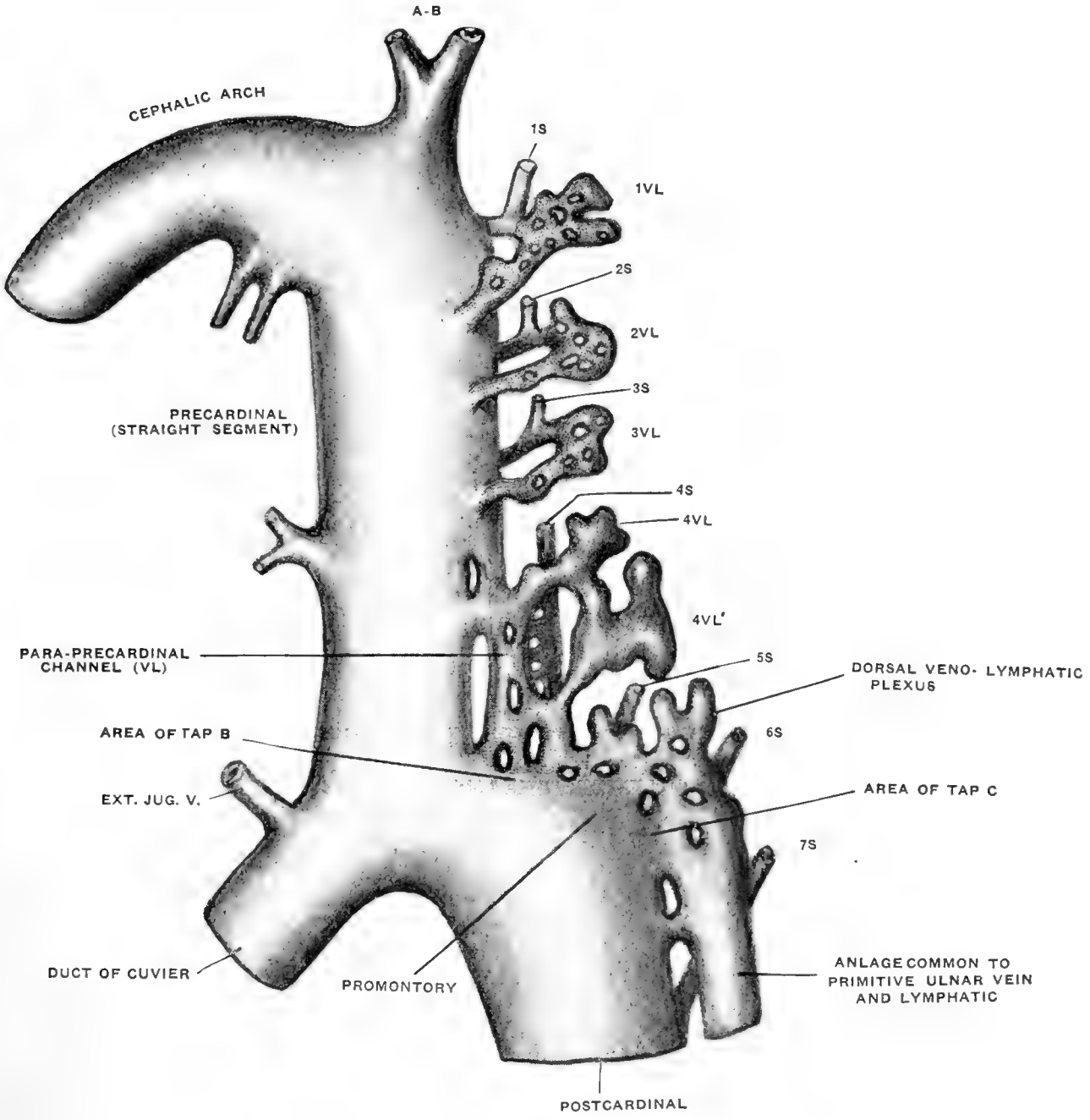

THYRO-CERVICAL A.
THORACIC DUCT
SYMPATHETIC N.
SUBCLAVIAN APPROACH
LEFT CAROTID A.
COMMON JUGULAR CONFLUENCE (TAP)

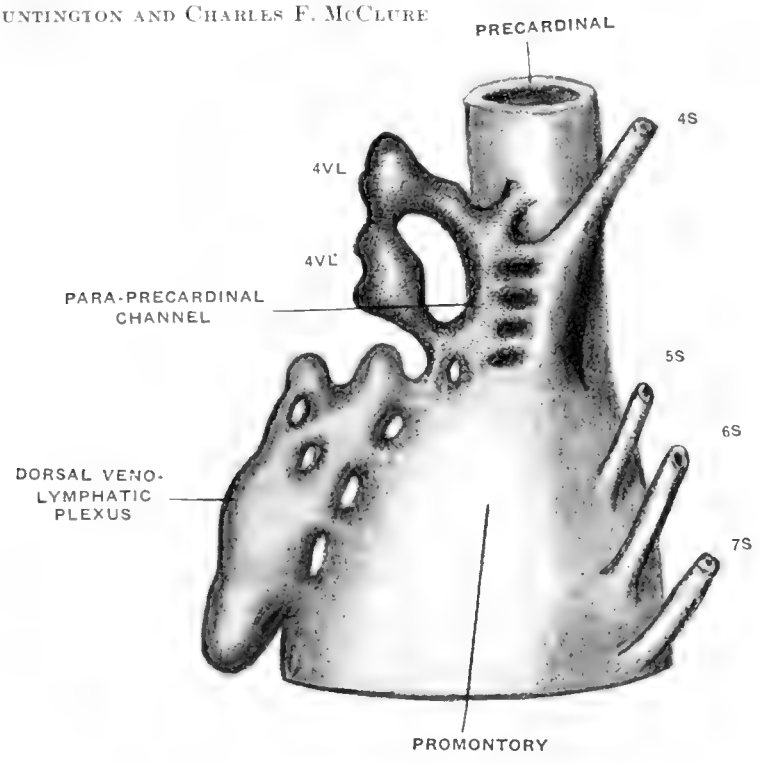
7


A-B
a' a'' a'''
b' b'' b'''
I ARCHED PORTION OF PRECARDINAL
1
II CAUDAL OR STRAIGHT SEGMENT OF PRECARDINAL
2
a
3
4S
4
b
5S
EXT. JUG. V.
DORSAL VENO-LYMPHATIC PLEXUS
III JUGULAR PROMONTORY AND DUCT OF CUVIER
6S
7S
8S
IV PRIMITIVE ULNAR SEGMENT OF POSTCARDINAL
ANLAGE OF PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
DUCT OF CUVIER
POSTCARDINAL
AREA OF CONFLUENCE BETWEEN DUCT OF CUVIER AND POSTCARDINAL

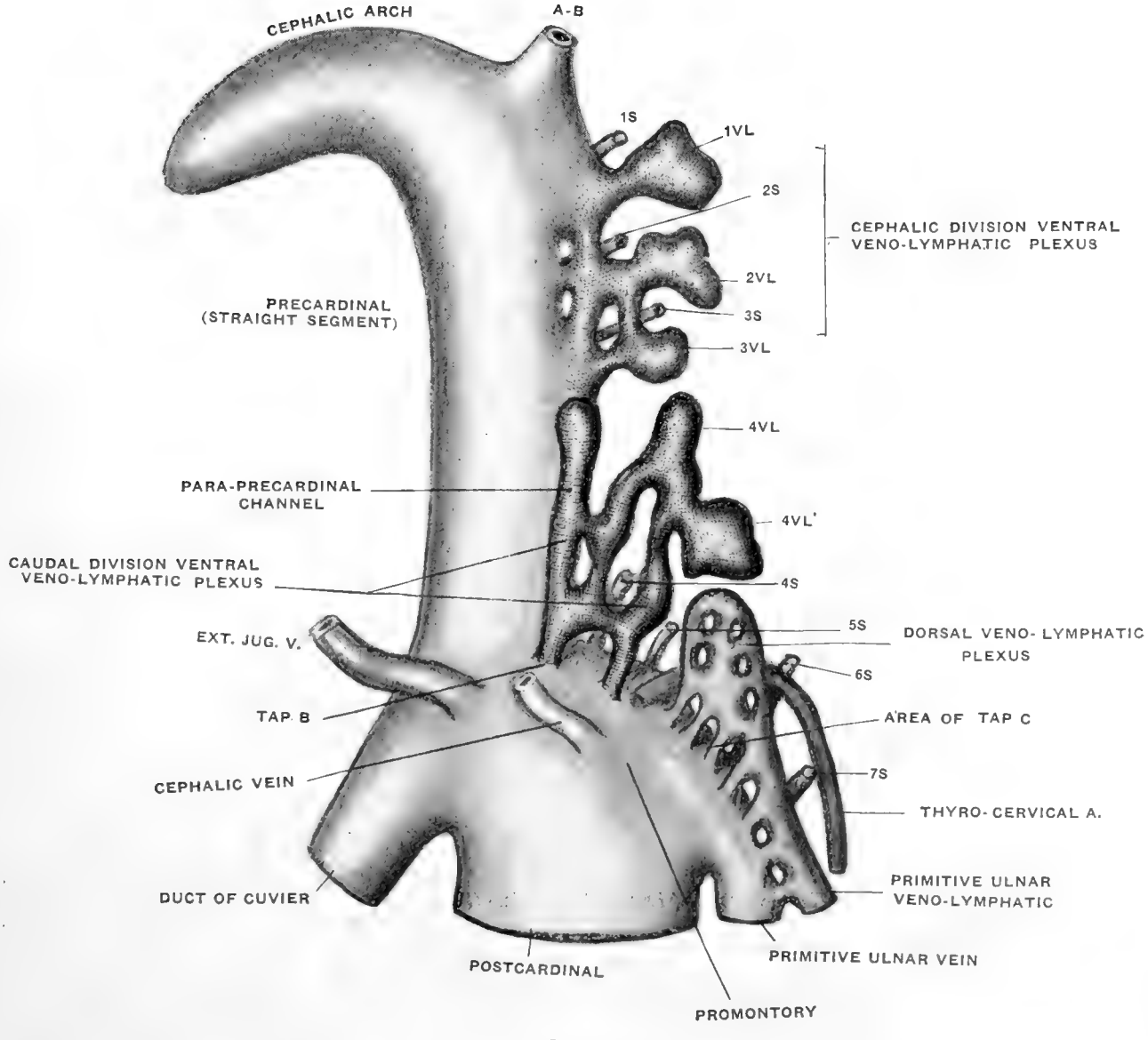

A-B
CEPHALIC ARCH
TAP A
1
2
CEPHALIC DIVISION VENTRAL
VENO-LYMPHATIC PLEXUS
PRECARDINAL
(STRAIGHT SEGMENT)
3
4S
4
PARA-PRECARDINAL
CHANNEL
5S
AREA OF TAP B
DORSAL VENO-
LYMPHATIC PLEXUS
6S
AREA OF TAP C
7S
DUCT OF CUVIER
PROMONTORY
ANLAGE COMMON TO
PRIMITIVE ULNAR VEIN
AND LYMPHATIC
POSTCARDINAL


A-B
CEPHALIC ARCH
1
2
3
PRECARDINAL
STRAIGHT SEGMENT
4S
4VL
4VL
PARA-PRECARDINAL
CHANNEL
5S
DORSAL VENO-
LYMPHATIC PLEXUS
6S
EXT. JUG. V.
AREA OF TAP C
7S
AREA OF TAP B
COMMON JUGULAR
LYMPH SAC
DUCT OF CUVIER
PROMONTORY
POSTCARDINAL

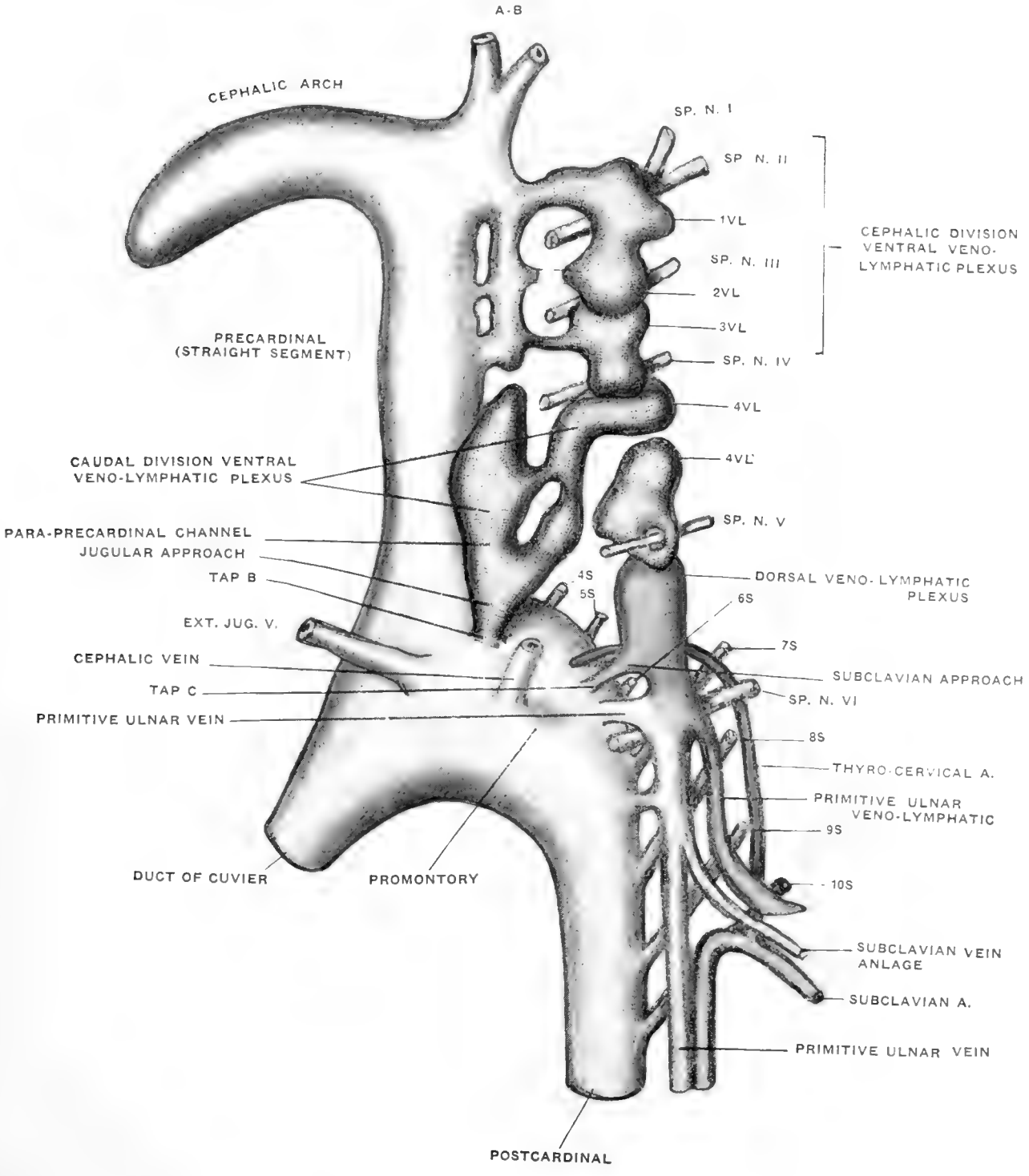

A-B
CEPHALIC ARCH
1S
1VL
2S
2VL
3S
3VL
PRECARDINAL
(STRAIGHT SEGMENT)
4S
4VL
4VL'
PARA-PRECARDINAL
CHANNEL (VL)
5S
DORSAL VENO- LYMPHATIC
PLEXUS
AREA OF TAP B
6S
EXT. JUG. V.
AREA OF TAP C
7S
DUCT OF CUVIER
PROMONTORY
ANLAGE COMMON TO
PRIMITIVE ULNAR VEIN
AND LYMPHATIC
POSTCARDINAL

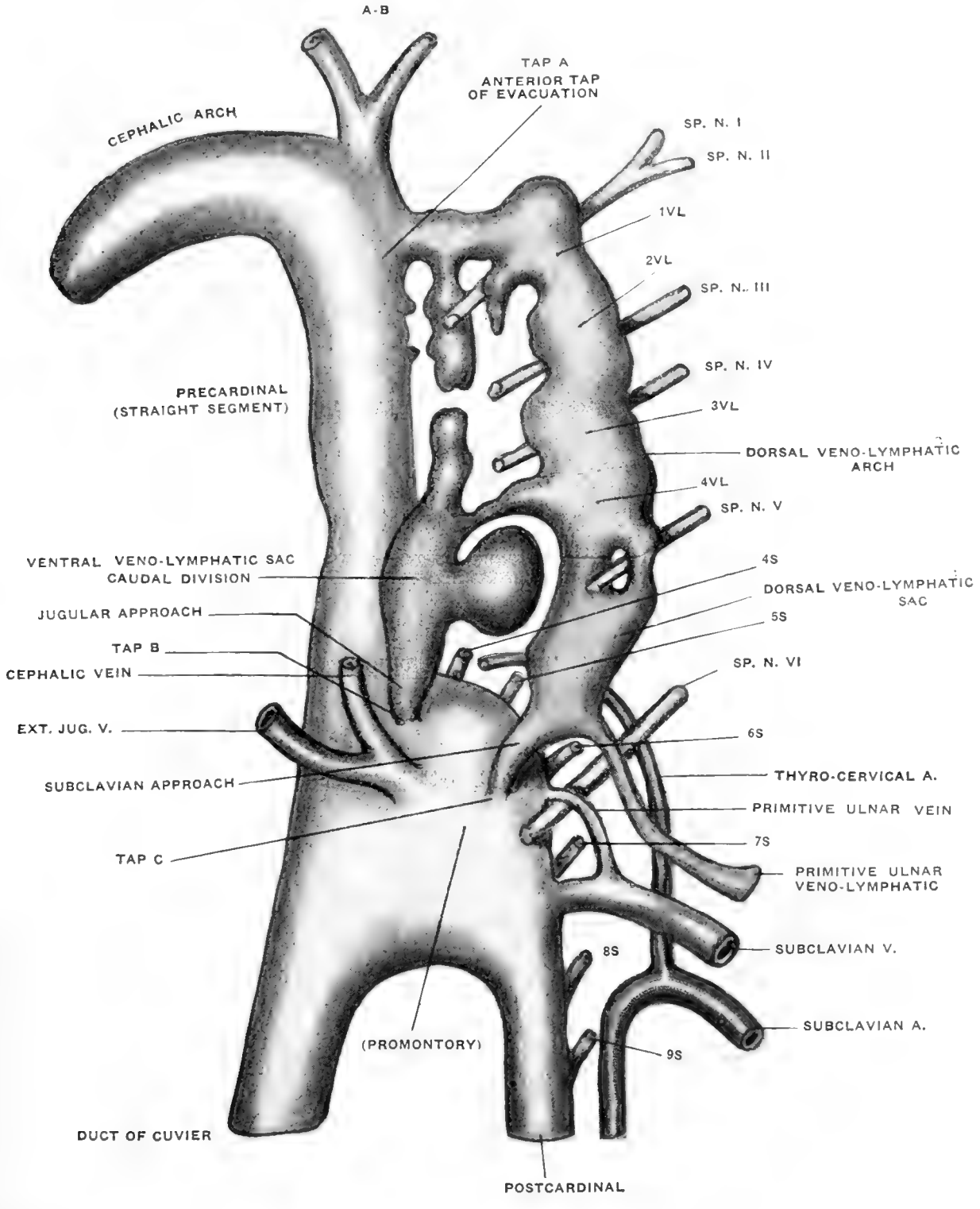

PRECARDINAL
4S
4VL
4VL'
PARA-PRECARDINAL CHANNEL
5S
6S
DORSAL VENO-LYMPHATIC PLEXUS
7S
PROMONTORY

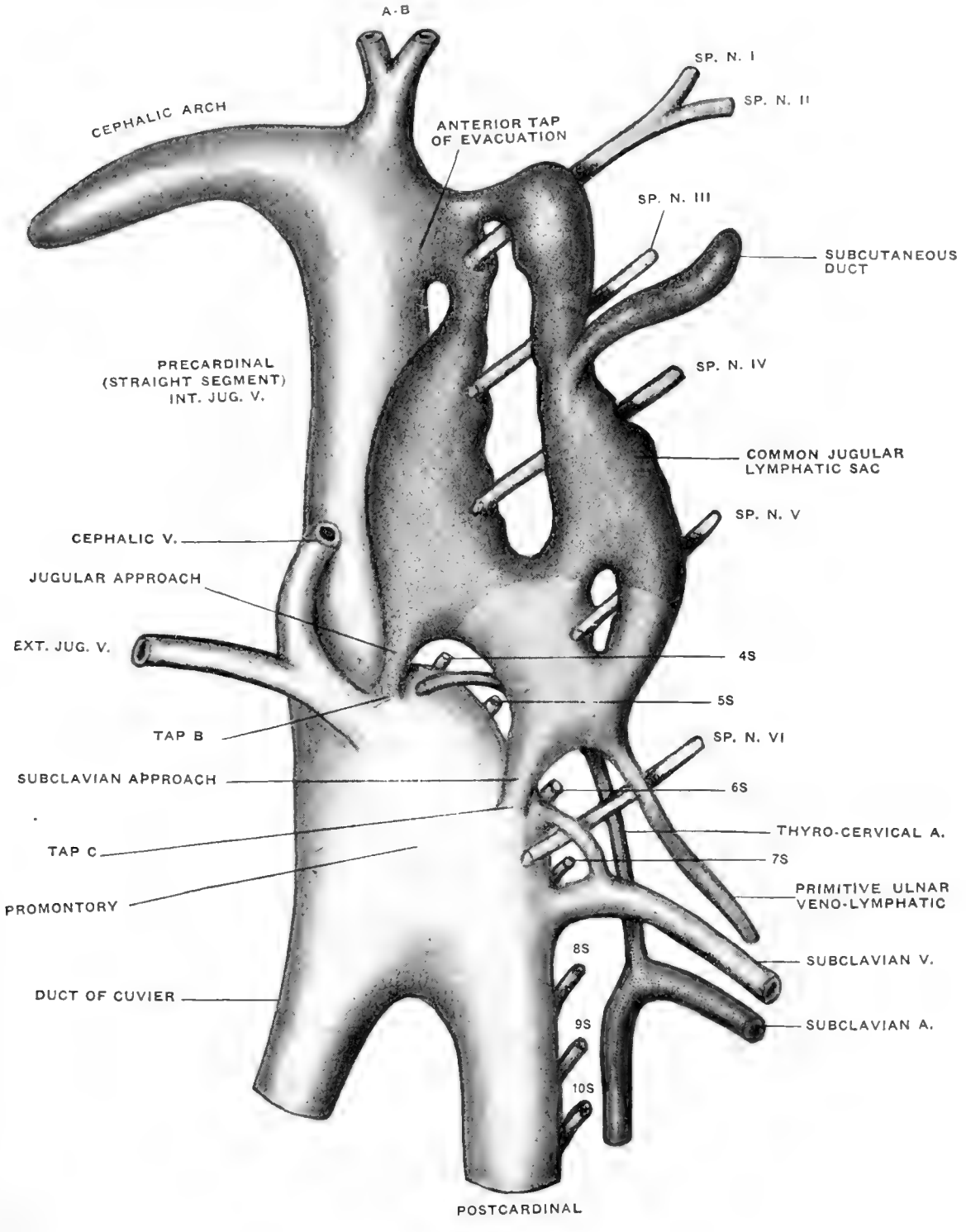
11A

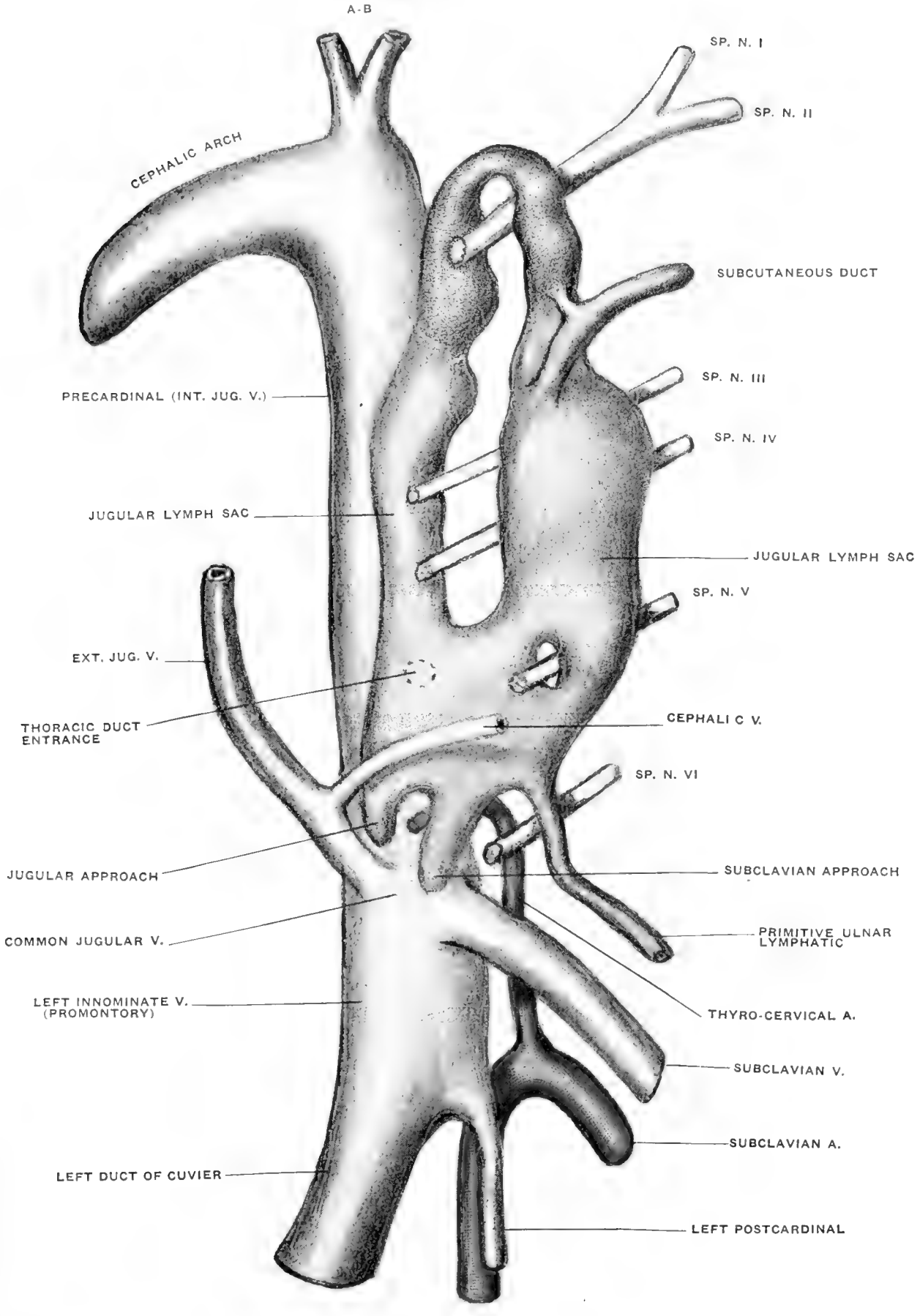

CEPHALIC ARCH
A-B
1S
1VL
2S
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
2VL
PRECARDINAL (STRAIGHT SEGMENT)
3S
3VL
4VL
PARA-PRECARDINAL CHANNEL
4VL'
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
4S
5S
DORSAL VENO-LYMPHATIC PLEXUS
EXT. JUG. V.
6S
TAP B
AREA OF TAP C
CEPHALIC VEIN
7S
THYRO-CERVICAL A.
PRIMITIVE ULNAR VENO-LYMPHATIC
DUCT OF CUVIER
PRIMITIVE ULNAR VEIN
POSTCARDINAL
PROMONTORY

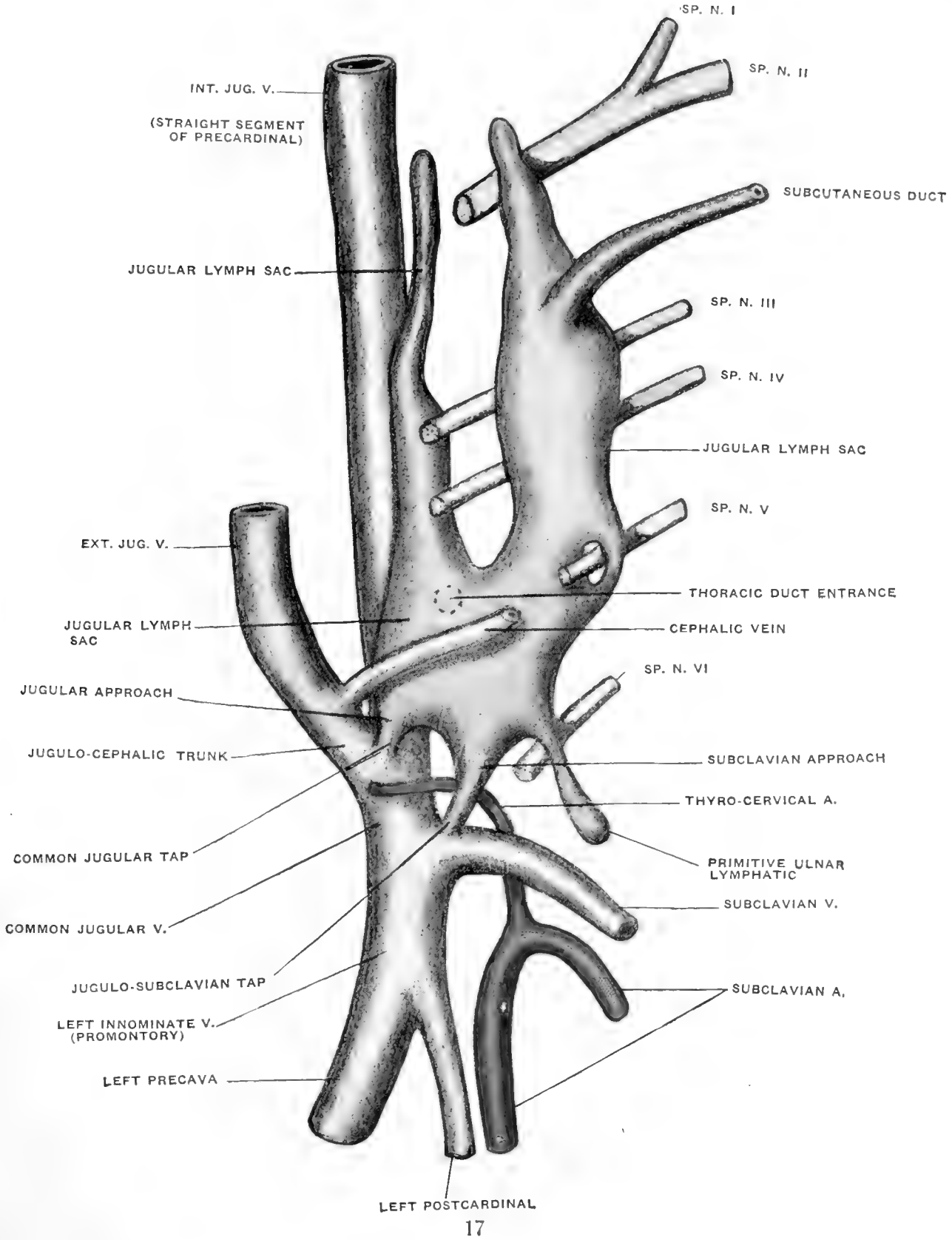
12

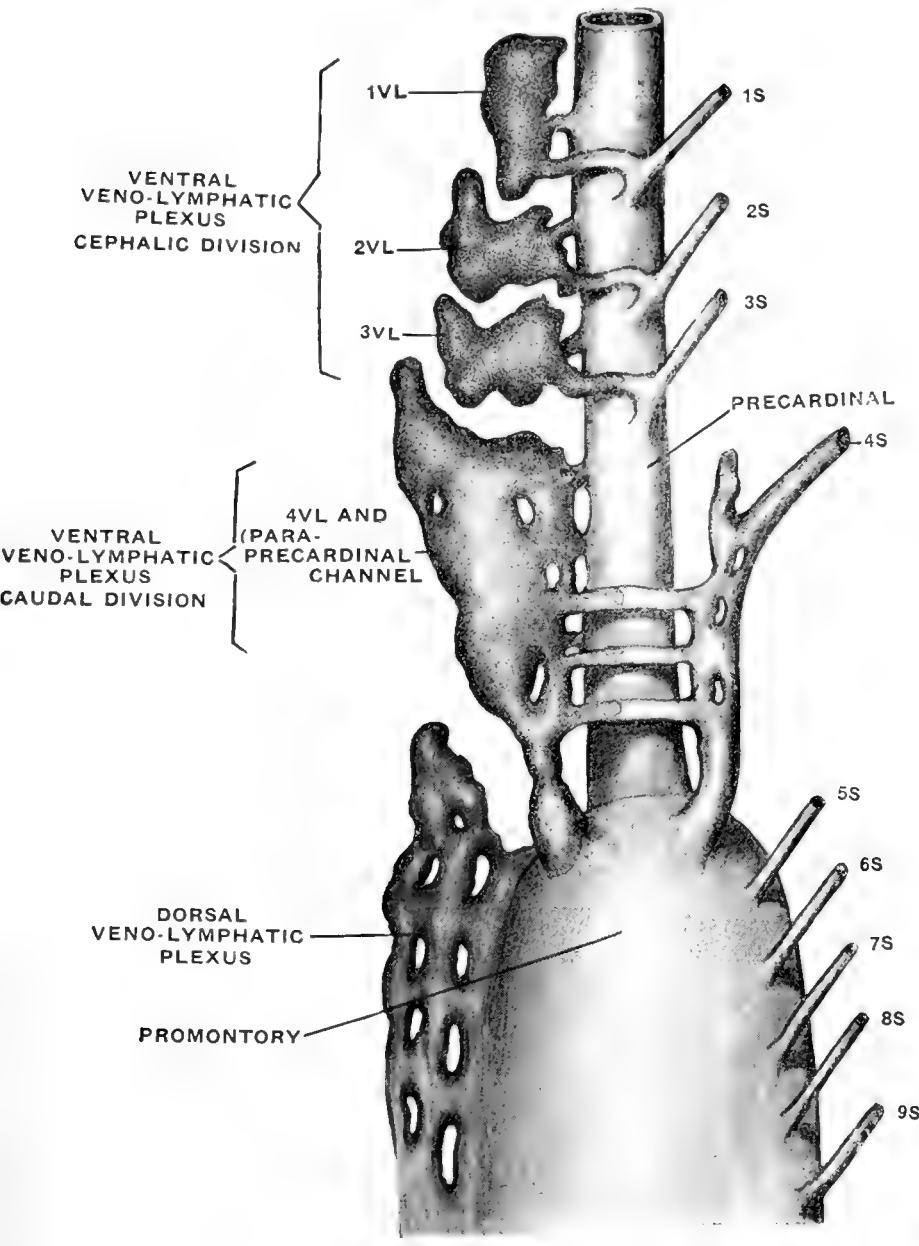

A-B
CEPHALIC ARCH
SP. N. I
SP. N. II
1VL
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SP. N. III
2VL
3VL
PRECARDINAL (STRAIGHT SEGMENT)
SP. N. IV
4VL
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
4VL'
SP. N. V
PARA-PRECARDINAL CHANNEL
JUGULAR APPROACH
TAP B
4S
5S
DORSAL VENO-LYMPHATIC PLEXUS
6S
EXT. JUG. V.
7S
CEPHALIC VEIN
SUBCLAVIAN APPROACH
TAP C
SP. N. VI
PRIMITIVE ULNAR VEIN
8S
THYRO-CERVICAL A.
PRIMITIVE ULNAR VENO-LYMPHATIC
9S
10S
DUCT OF CUVIER
PROMONTORY
SUBCLAVIAN VEIN ANLAGE
SUBCLAVIAN A.
PRIMITIVE ULNAR VEIN
POSTCARDINAL


A-B
TAP A
ANTERIOR TAP
OF EVACUATION
CEPHALIC ARCH
SP. N. I
SP. N. II
1VL
2VL
SP. N. III
SP. N. IV
PRECARDINAL
(STRAIGHT SEGMENT)
3VL
DORSAL VENO-LYMPHATIC
ARCH
4VL
SP. N. V
VENTRAL VENO-LYMPHATIC SAC
CAUDAL DIVISION
4S
DORSAL VENO-LYMPHATIC
SAC
JUGULAR APPROACH
5S
TAP B
CEPHALIC VEIN
SP. N. VI
EXT. JUG. V.
6S
THYRO-CERVICAL A.
SUBCLAVIAN APPROACH
PRIMITIVE ULNAR VEIN
7S
TAP C
PRIMITIVE ULNAR
VENO-LYMPHATIC
8S
SUBCLAVIAN V.
SUBCLAVIAN A.
(PROMONTORY)
9S
DUCT OF CUVIER
POSTCARDINAL

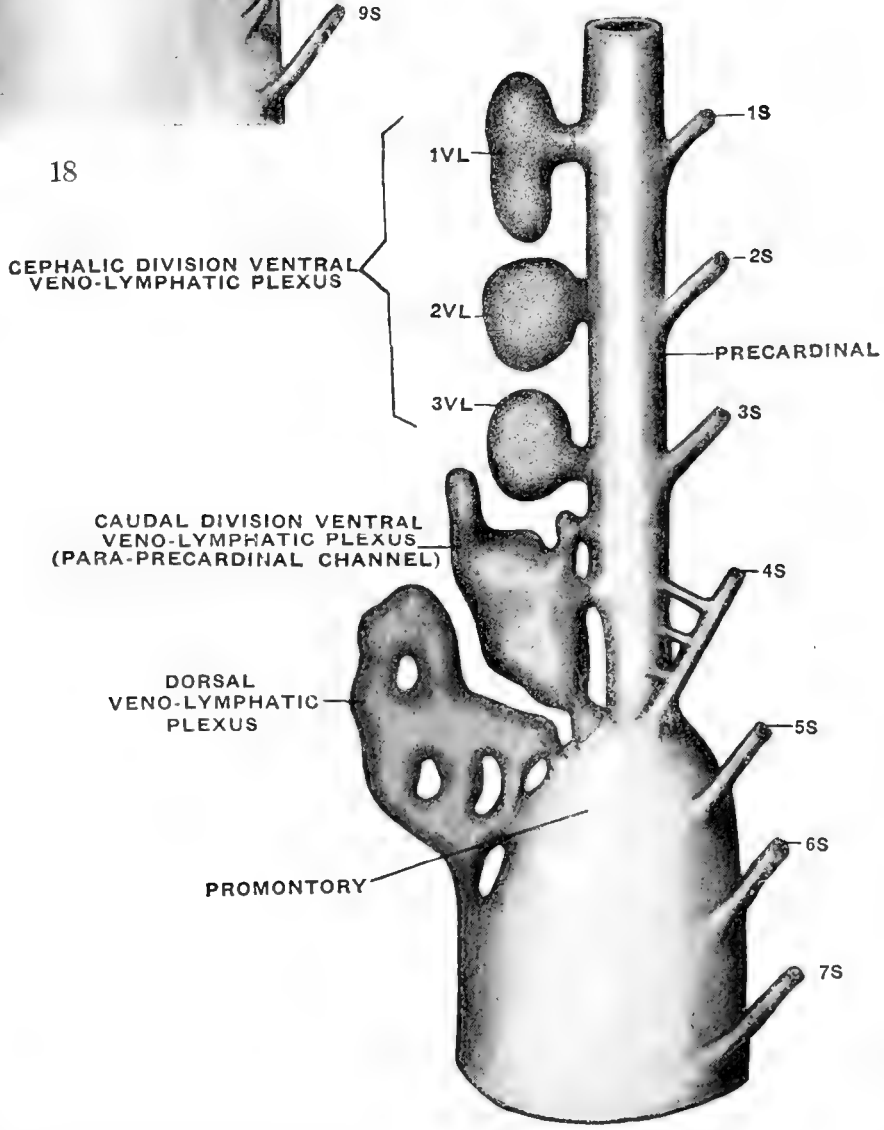

A-B
SP. N. I
SP. N. II
CEPHALIC ARCH
ANTERIOR TAP OF EVACUATION
SP. N. III
SUBCUTANEOUS DUCT
PRECARDINAL (STRAIGHT SEGMENT) INT. JUG. V.
SP. N. IV
COMMON JUGULAR LYMPHATIC SAC
SP. N. V
CEPHALIC V.
JUGULAR APPROACH
EXT. JUG. V.
4S
5S
TAP B
SP. N. VI
SUBCLAVIAN APPROACH
6S
THYRO-CERVICAL A.
TAP C
7S
PRIMITIVE ULNAR VENO-LYMPHATIC
PROMONTORY
8S
SUBCLAVIAN V.
DUCT OF CUVIER
9S
SUBCLAVIAN A.
10S
POSTCARDINAL


A-B
SP. N. I
SP. N. II
CEPHALIC ARCH
SUBCUTANEOUS DUCT
SP. N. III
PRECARDINAL (INT. JUG. V.)
SP. N. IV
JUGULAR LYMPH SAC
JUGULAR LYMPH SAC
SP. N. V
EXT. JUG. V.
THORACIC DUCT ENTRANCE
CEPHALI C V.
SP. N. VI
JUGULAR APPROACH
SUBCLAVIAN APPROACH
COMMON JUGULAR V.
PRIMITIVE ULNAR LYMPHATIC
LEFT INNOMINATE V. (PROMONTORY)
THYRO-CERVICAL A.
SUBCLAVIAN V.
SUBCLAVIAN A.
LEFT DUCT OF CUVIER
LEFT POSTCARDINAL

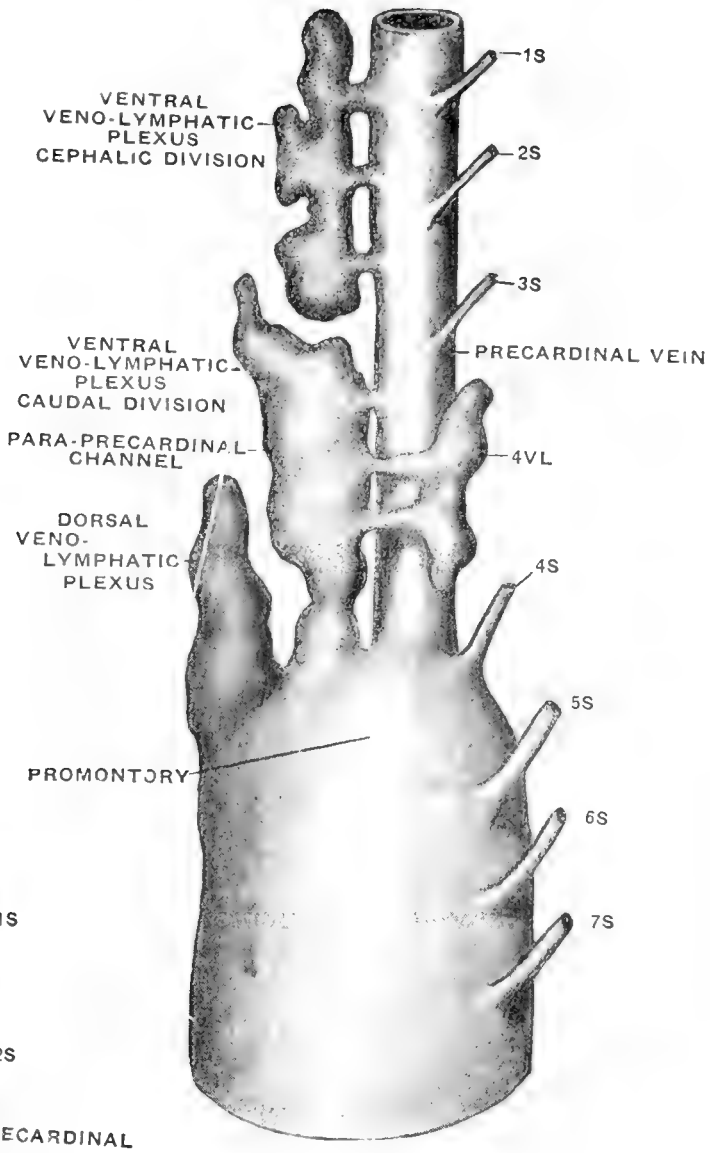

SP. N. I
SP. N. II
INT. JUG. V.
(STRAIGHT SEGMENT OF PRECARDINAL)
SUBCUTANEOUS DUCT
JUGULAR LYMPH SAC
SP. N. III
SP. N. IV
JUGULAR LYMPH SAC
SP. N. V
EXT. JUG. V.
THORACIC DUCT ENTRANCE
JUGULAR LYMPH SAC
CEPHALIC VEIN
SP. N. VI
JUGULAR APPROACH
JUGULO-CEPHALIC TRUNK
SUBCLAVIAN APPROACH
THYRO-CERVICAL A.
COMMON JUGULAR TAP
PRIMITIVE ULNAR LYMPHATIC
SUBCLAVIAN V.
COMMON JUGULAR V.
JUGULO-SUBCLAVIAN TAP
SUBCLAVIAN A.
LEFT INNOMINATE V. (PROMONTORY)
LEFT PRECAVA
LEFT POSTCARDINAL


1VL
1S
VENTRAL VENO-LYMPHATIC PLEXUS CEPHALIC DIVISION
2VL
2S
3VL
3S
PRECARDINAL
4S
VENTRAL VENO-LYMPHATIC PLEXUS CAUDAL DIVISION
4VL AND (PARA-PRECARDINAL CHANNEL
5S
6S
DORSAL VENO-LYMPHATIC PLEXUS
7S
PROMONTORY
8S
9S

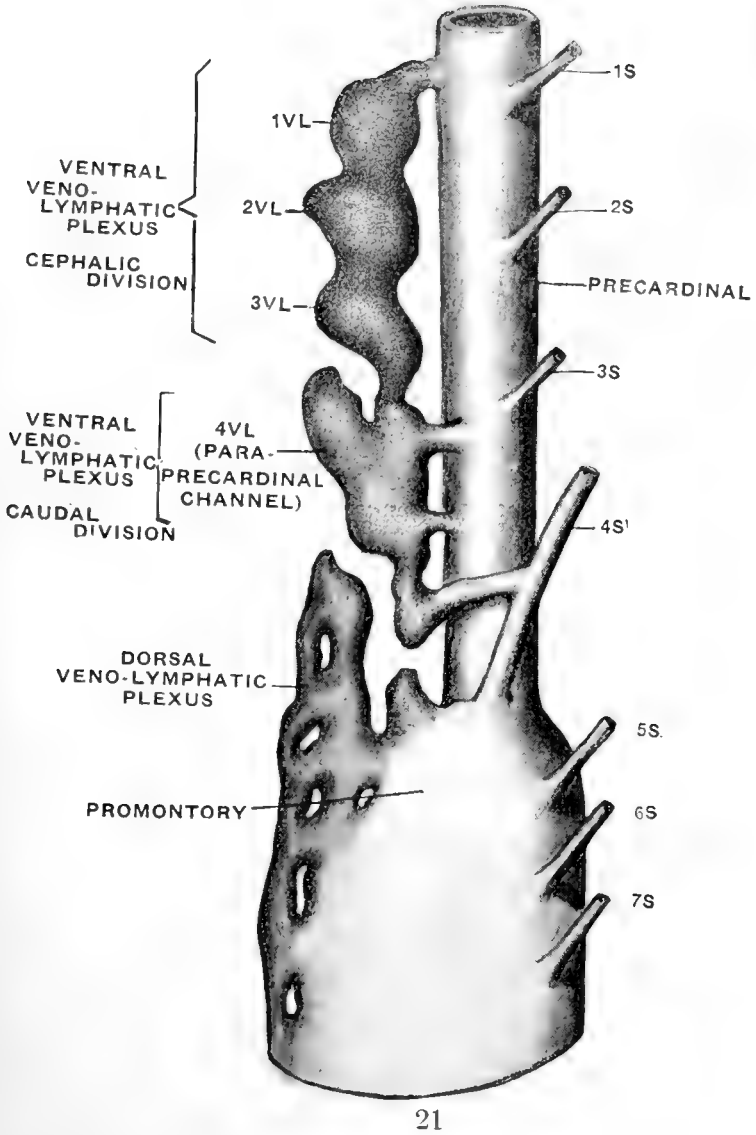
18


1S
1VL
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
2S
2VL
PRECARDINAL
3VL
3S
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS (PARA-PRECARDINAL CHANNEL)
4S
DORSAL VENO-LYMPHATIC PLEXUS
5S
6S
PROMONTORY
7S

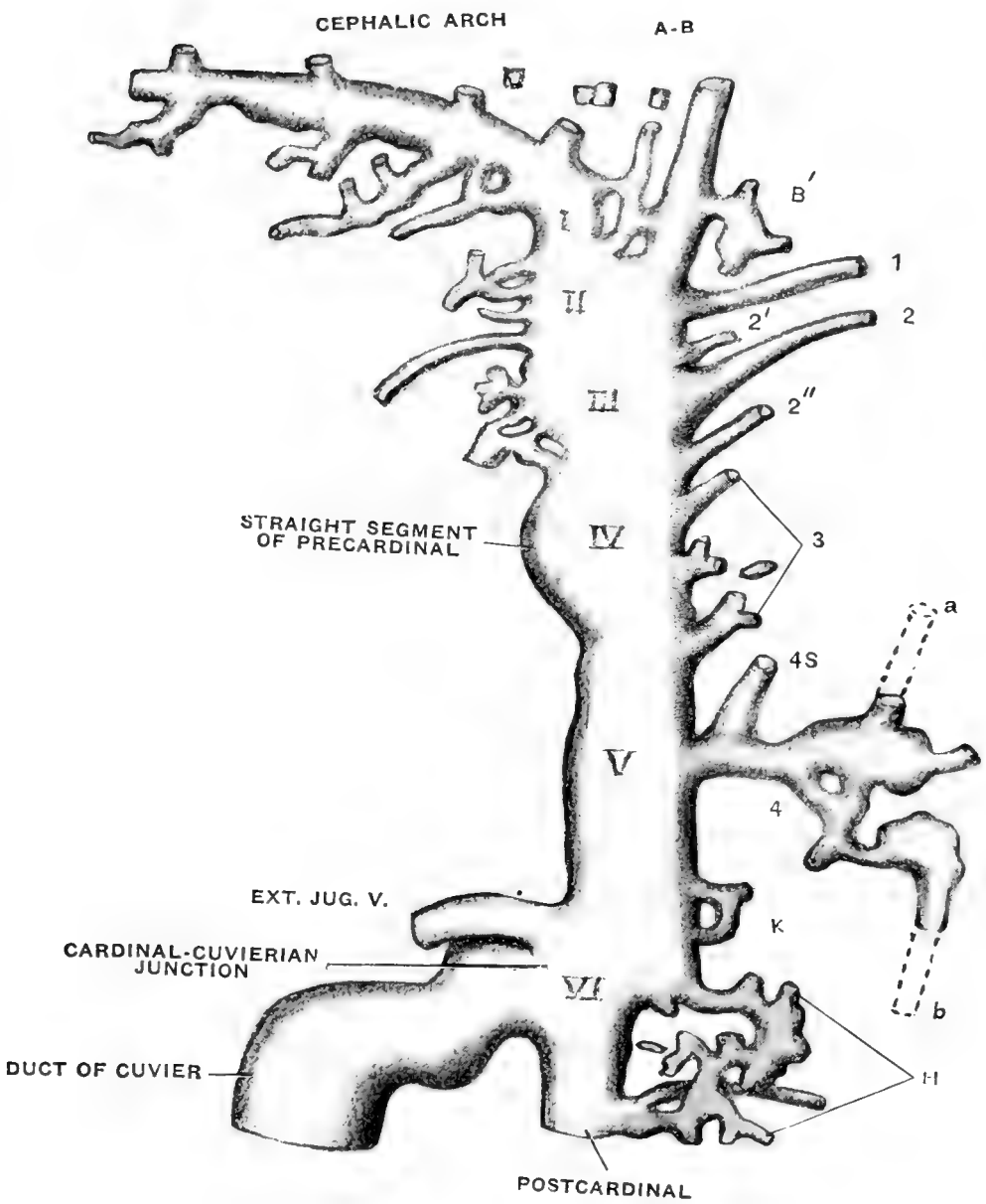
19

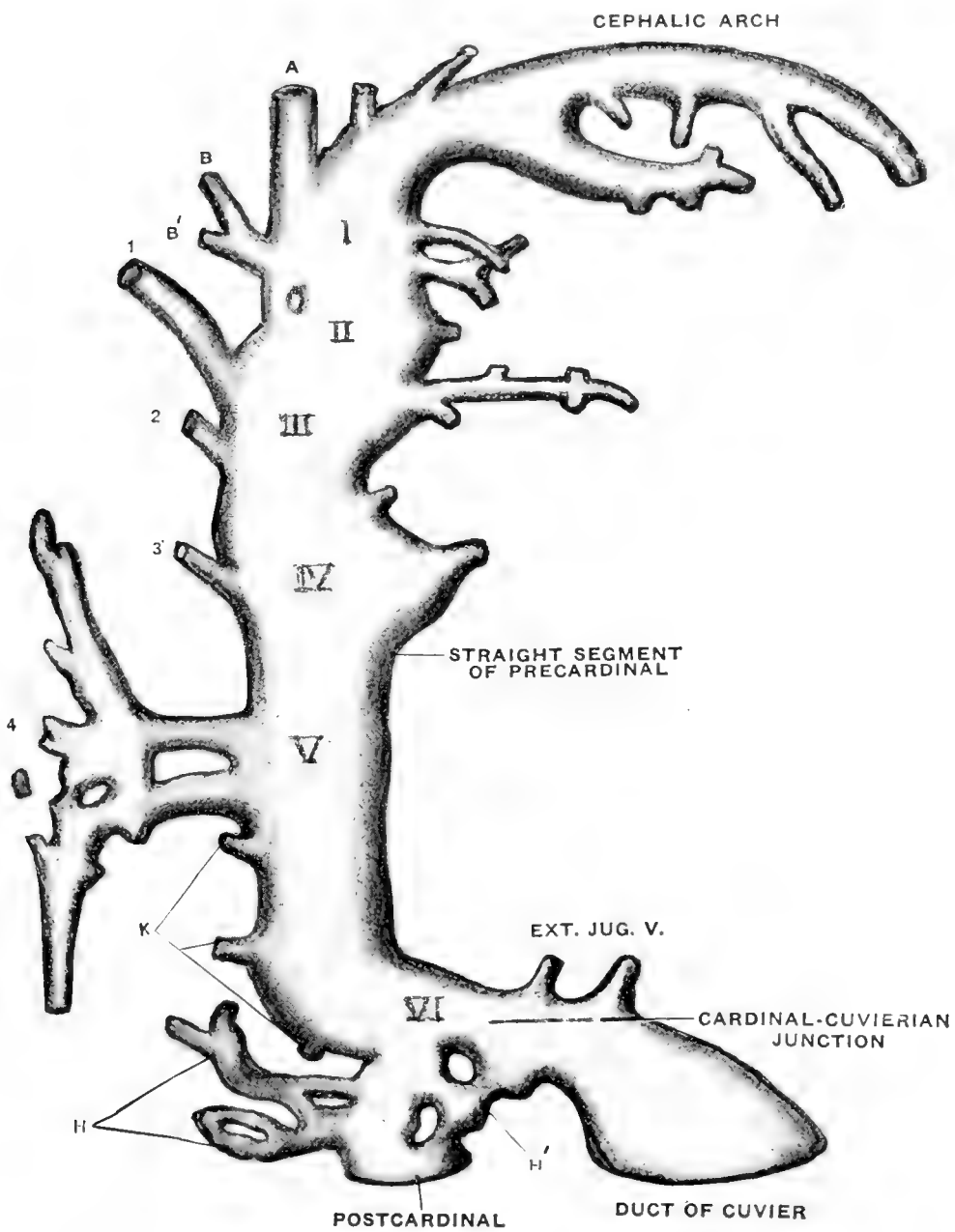

1S
VENTRAL VENO-LYMPHATIC PLEXUS CEPHALIC DIVISION
2S
3S
PRECARDINAL VEIN
VENTRAL VENO-LYMPHATIC PLEXUS CAUDAL DIVISION
PARA-PRECARDINAL CHANNEL
4VL
DORSAL VENO-LYMPHATIC PLEXUS
4S
5S
PROMONTORY
6S
7S

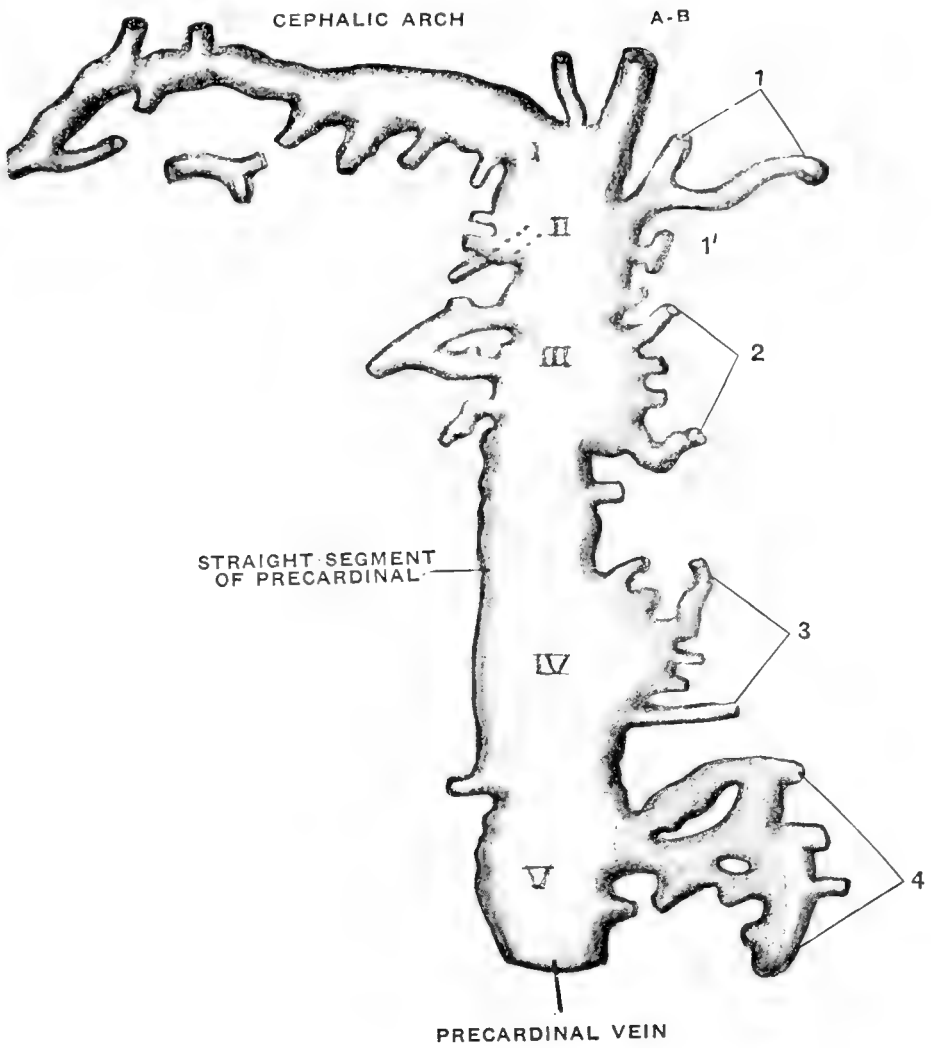
20


1S
1VL
VENTRAL VENO-LYMPHATIC PLEXUS
2VL
2S
CEPHALIC DIVISION
PRECARDINAL
3VL
3S
VENTRAL VENO-LYMPHATIC PLEXUS
4VL (PARA-PRECARDINAL CHANNEL)
CAUDAL DIVISION
4S
DORSAL VENO-LYMPHATIC PLEXUS
5S
PROMONTORY
6S
7S

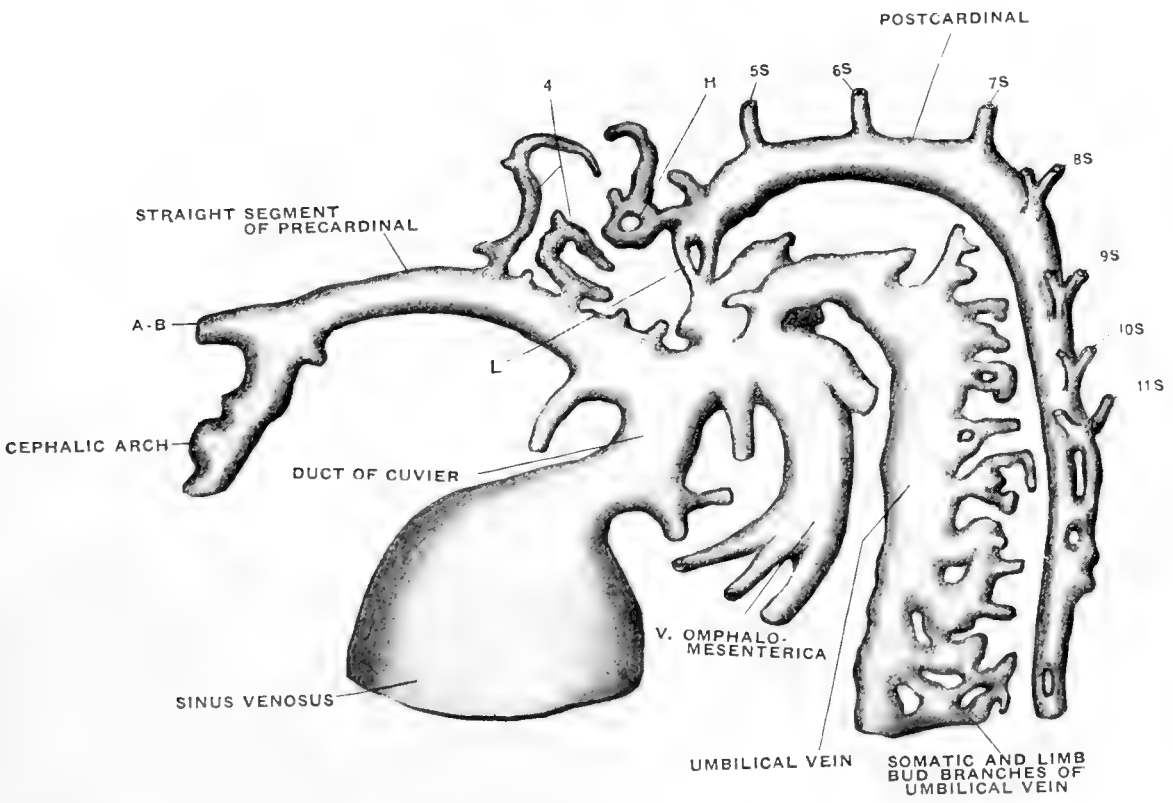
21


CEPHALIC ARCH
A-B
B′
1
2
2′
2″
3
4S
4
a
b
K
H
I
II
III
IV
V
VI
STRAIGHT SEGMENT OF PRECARDINAL
EXT. JUG. V.
CARDINAL-CUVIERIAN JUNCTION
DUCT OF CUVIER
POSTCARDINAL

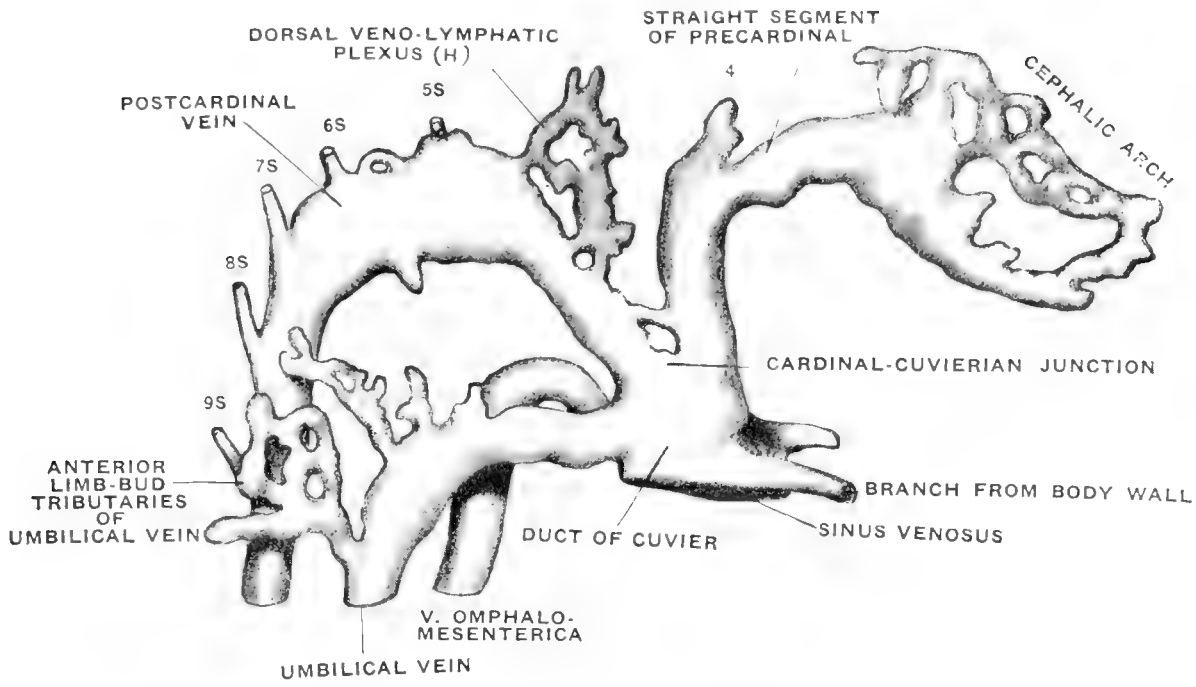

CEPHALIC ARCH
A
B
B'
1
2
3
4
I
II
III
IV
V
VI
STRAIGHT SEGMENT OF PRECARDINAL
K
EXT. JUG. V.
CARDINAL-CUVIERIAN JUNCTION
H
H'
POSTCARDINAL
DUCT OF CUVIER


CEPHALIC ARCH
A-B
1
I
II
1'
III
2
STRAIGHT SEGMENT OF PRECARDINAL
3
IV
V
4
PRECARDINAL VEIN

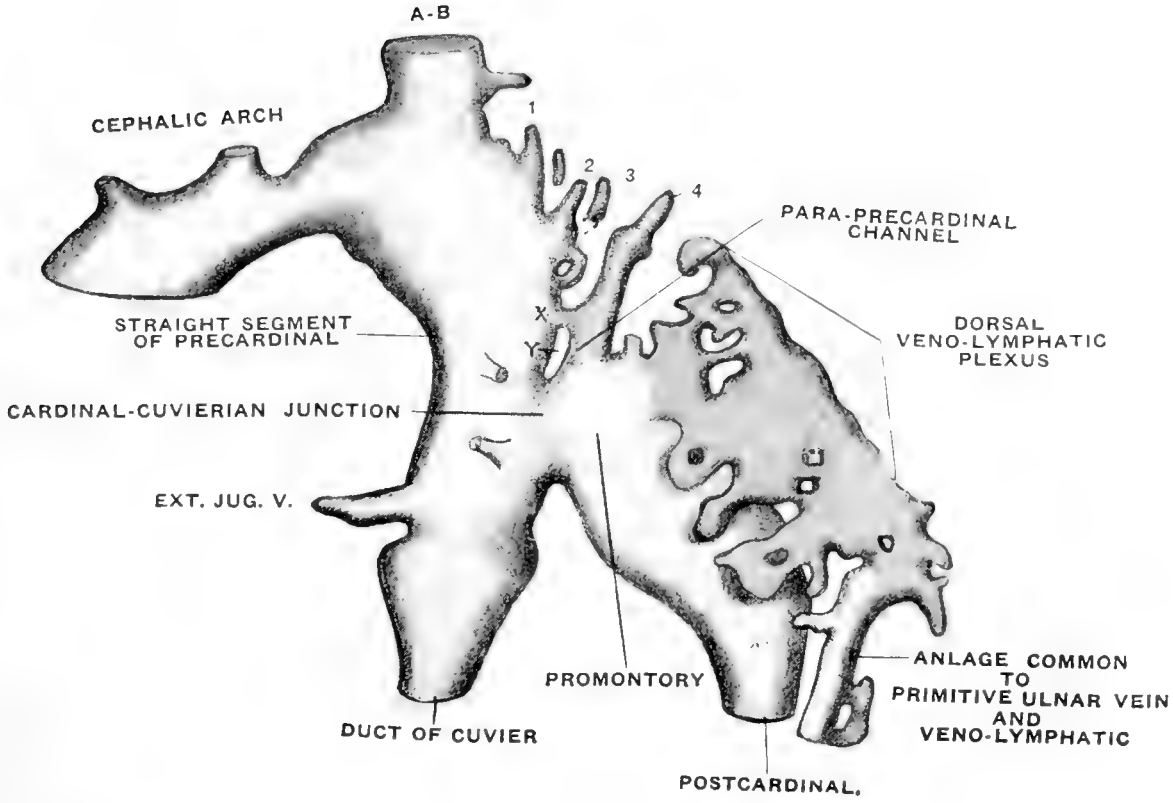
24


POSTCARDINAL
4
H
5S
6S
7S
8S
9S
10S
11S
STRAIGHT SEGMENT OF PRECARDINAL
A-B
L
CEPHALIC ARCH
DUCT OF CUVIER
V. OMPHALO-MESENTERICA
SINUS VENOSUS
UMBILICAL VEIN
SOMATIC AND LIMB BUD BRANCHES OF UMBILICAL VEIN

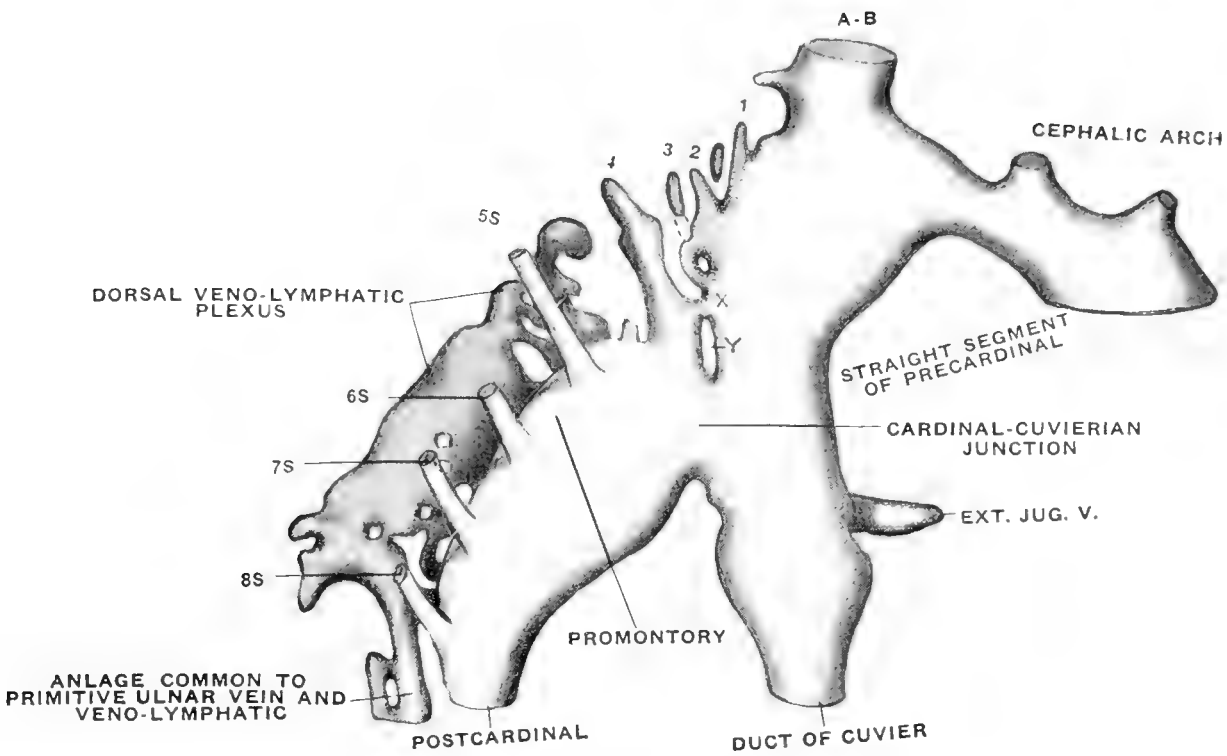
25


DORSAL VENO-LYMPHATIC PLEXUS (H)
STRAIGHT SEGMENT OF PRECARDINAL
POSTCARDINAL VEIN
5S
6S
7S
8S
9S
4
CEPHALIC ARCH
CARDINAL-CUVIERIAN JUNCTION
ANTERIOR LIMB-BUD TRIBUTARIES OF UMBILICAL VEIN
BRANCH FROM BODY WALL
DUCT OF CUVIER
SINUS VENOSUS
V. OMPHALO-MESENTERICA
UMBILICAL VEIN

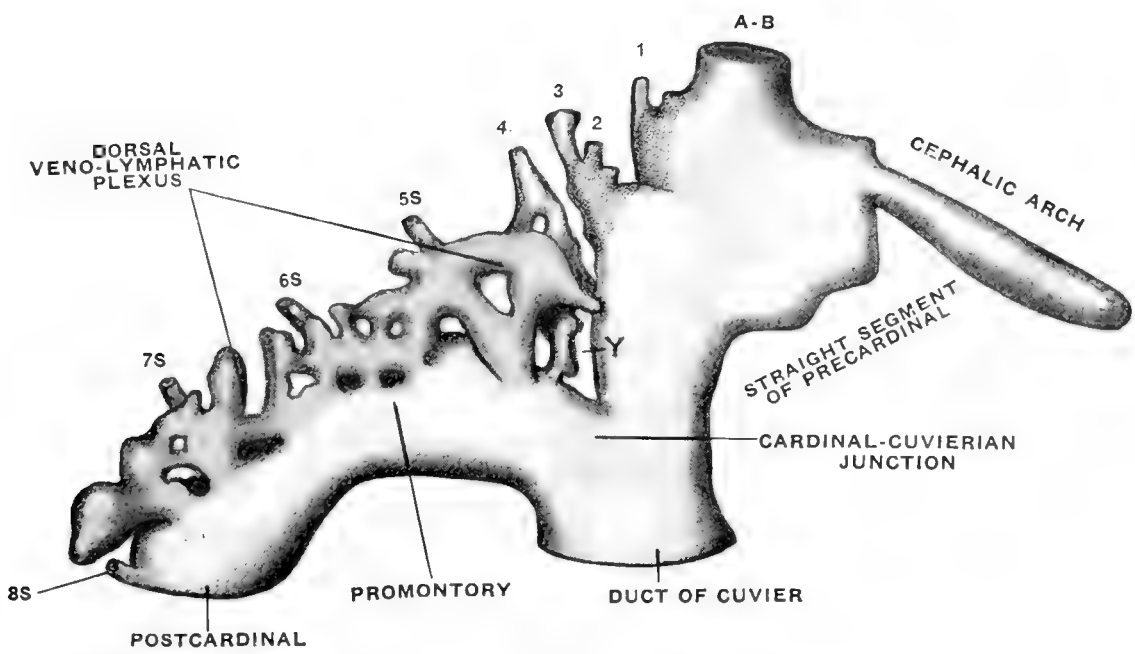
26


A-B
CEPHALIC ARCH
1
2
3
4
PARA-PRECARDINAL CHANNEL
STRAIGHT SEGMENT OF PRECARDINAL
X
Y
DORSAL VENO-LYMPHATIC PLEXUS
CARDINAL-CUVIERIAN JUNCTION
EXT. JUG. V.
PROMONTORY
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
DUCT OF CUVIER
POSTCARDINAL.

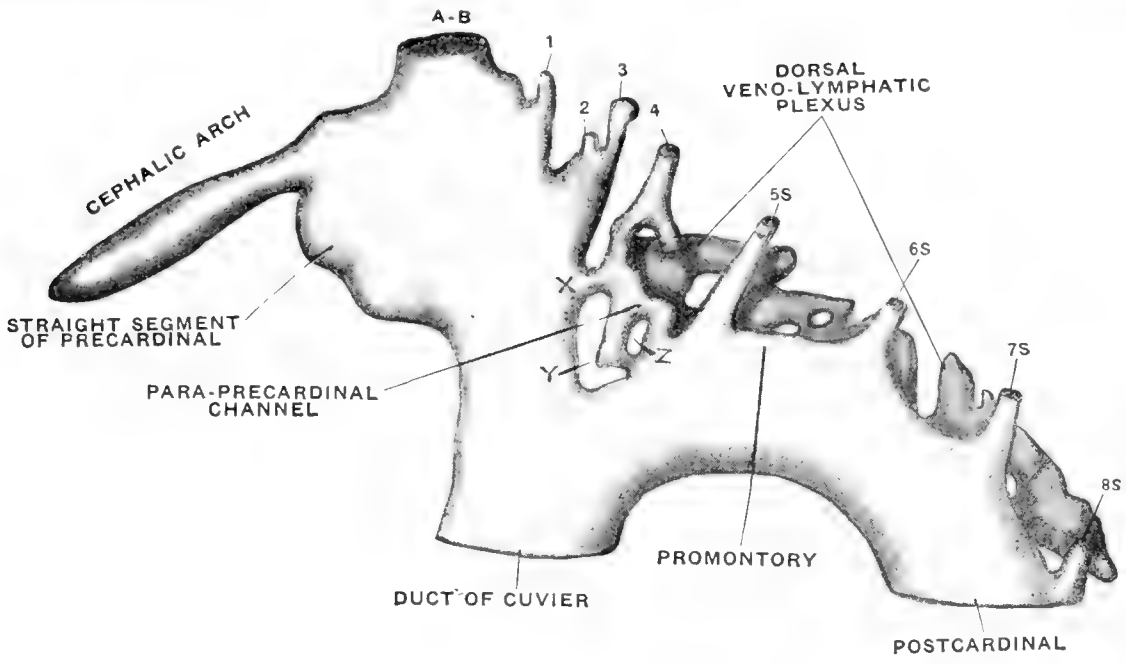
27


A-B
1
3
2
4
5S
CEPHALIC ARCH
DORSAL VENO-LYMPHATIC PLEXUS
X
Y
STRAIGHT SEGMENT OF PRECARDINAL
6S
CARDINAL-CUVIERIAN JUNCTION
7S
EXT. JUG. V.
8S
PROMONTORY
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
POSTCARDINAL
DUCT OF CUVIER

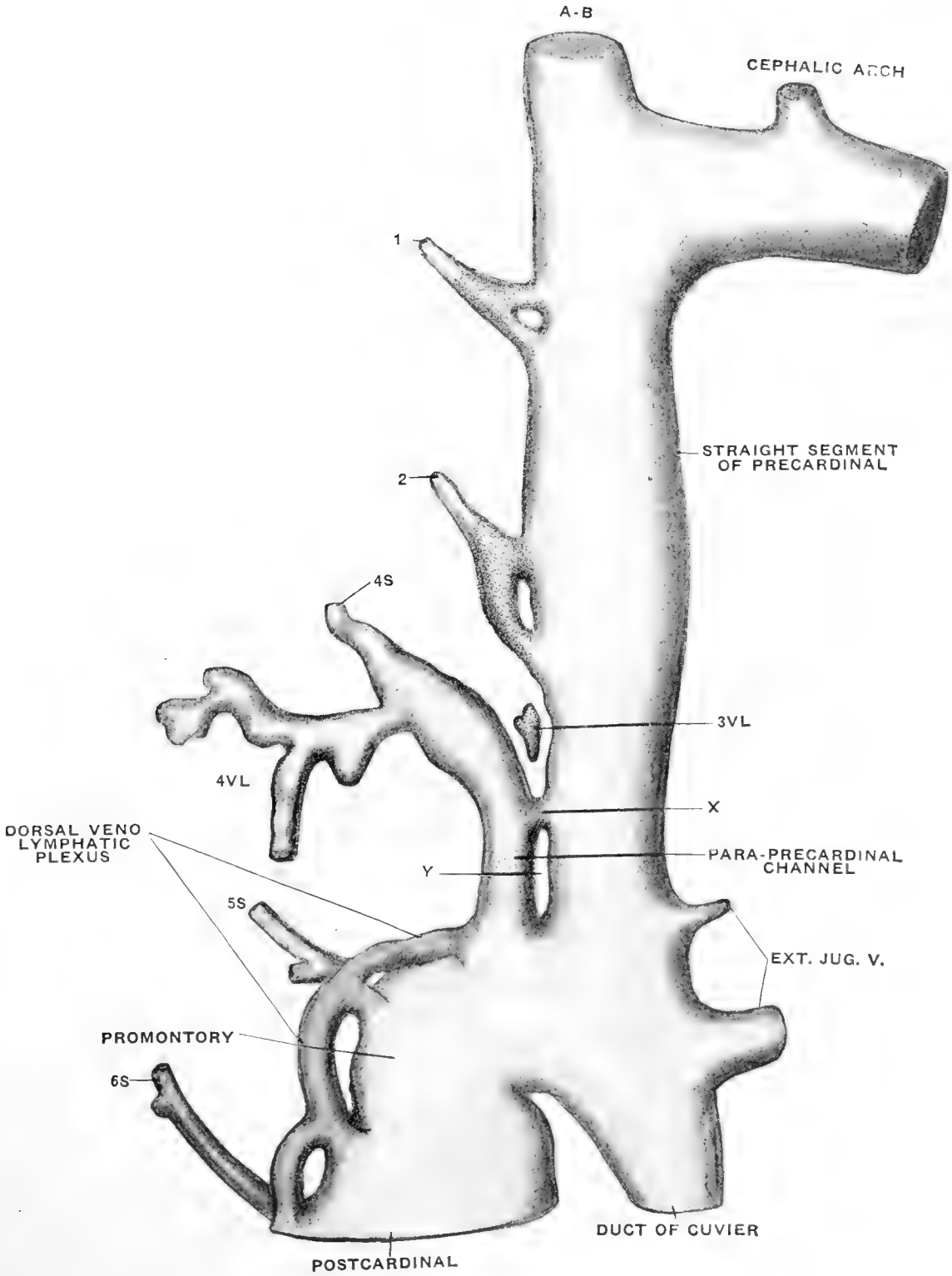
28


A-B
1
3
4
2
CEPHALIC ARCH
DORSAL VENO-LYMPHATIC PLEXUS
5S
6S
Y
7S
STRAIGHT SEGMENT OF PRECARDINAL
CARDINAL-CUVIERIAN JUNCTION
8S
PROMONTORY
DUCT OF CUVIER
POSTCARDINAL

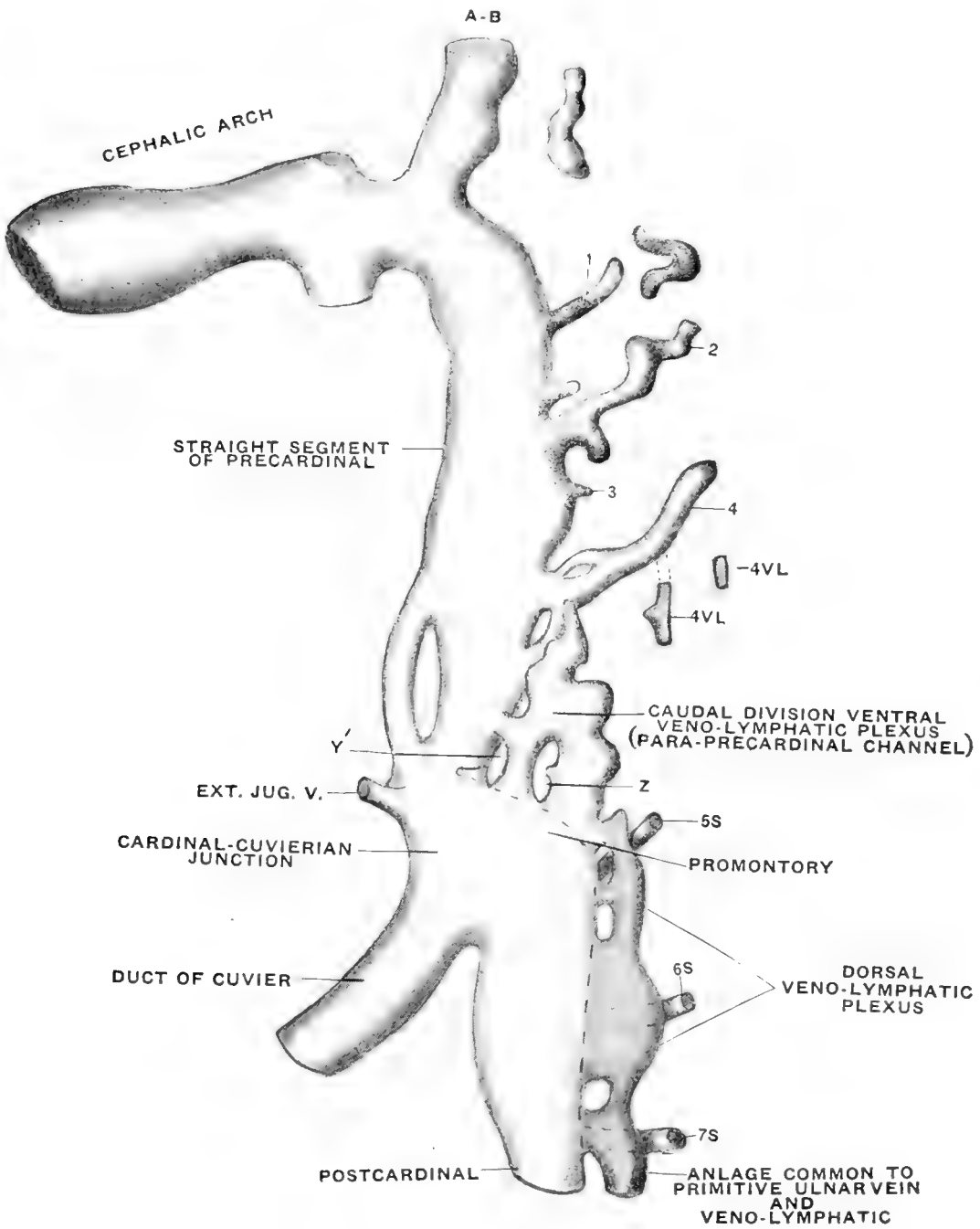
29

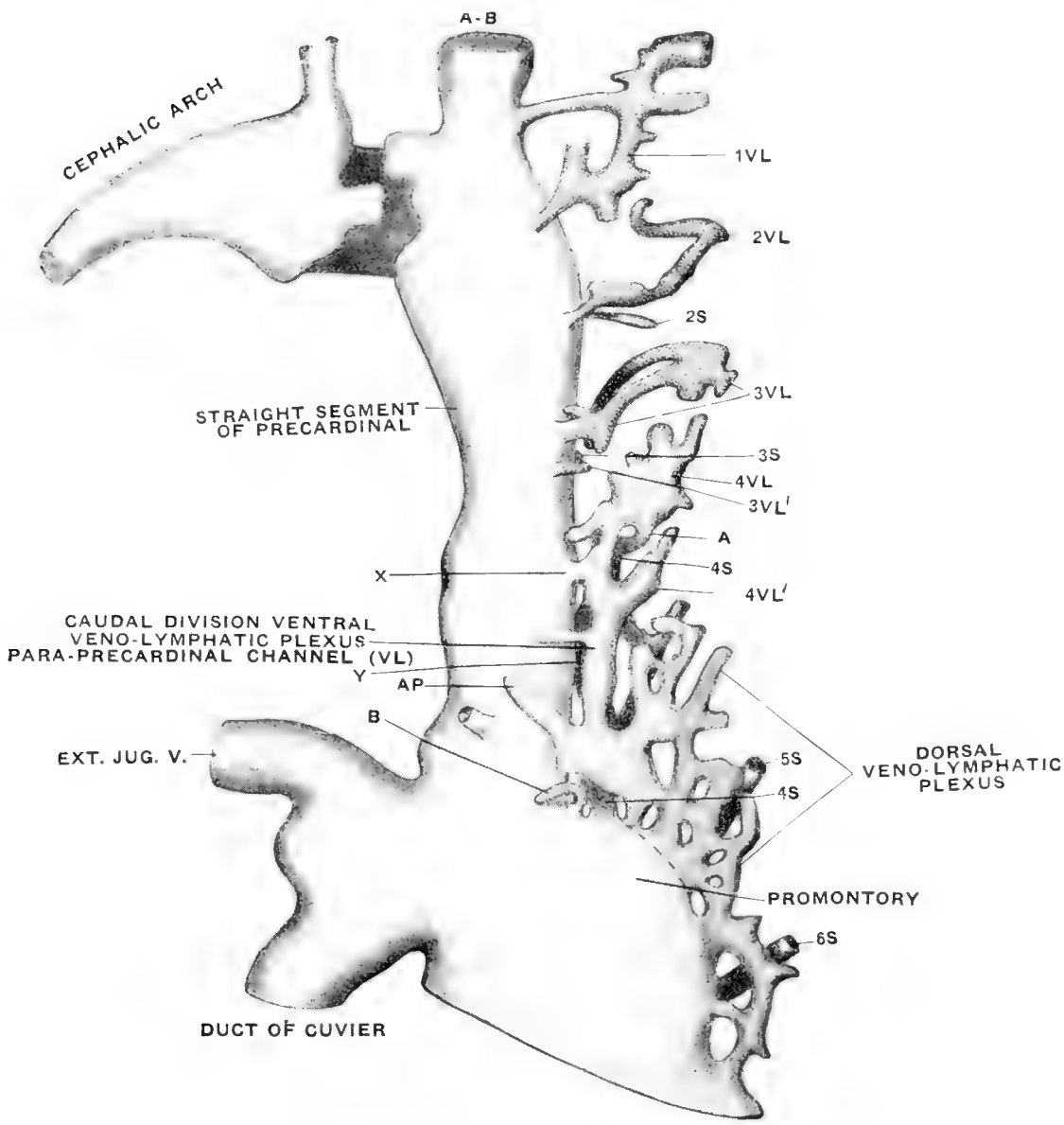

A-B
1
2
3
4
DORSAL
VENO-LYMPHATIC
PLEXUS
CEPHALIC ARCH
5S
6S
X
STRAIGHT SEGMENT
OF PRECARDINAL
Y
Z
7S
PARA-PRECARDINAL
CHANNEL
8S
PROMONTORY
DUCT OF CUVIER
POSTCARDINAL

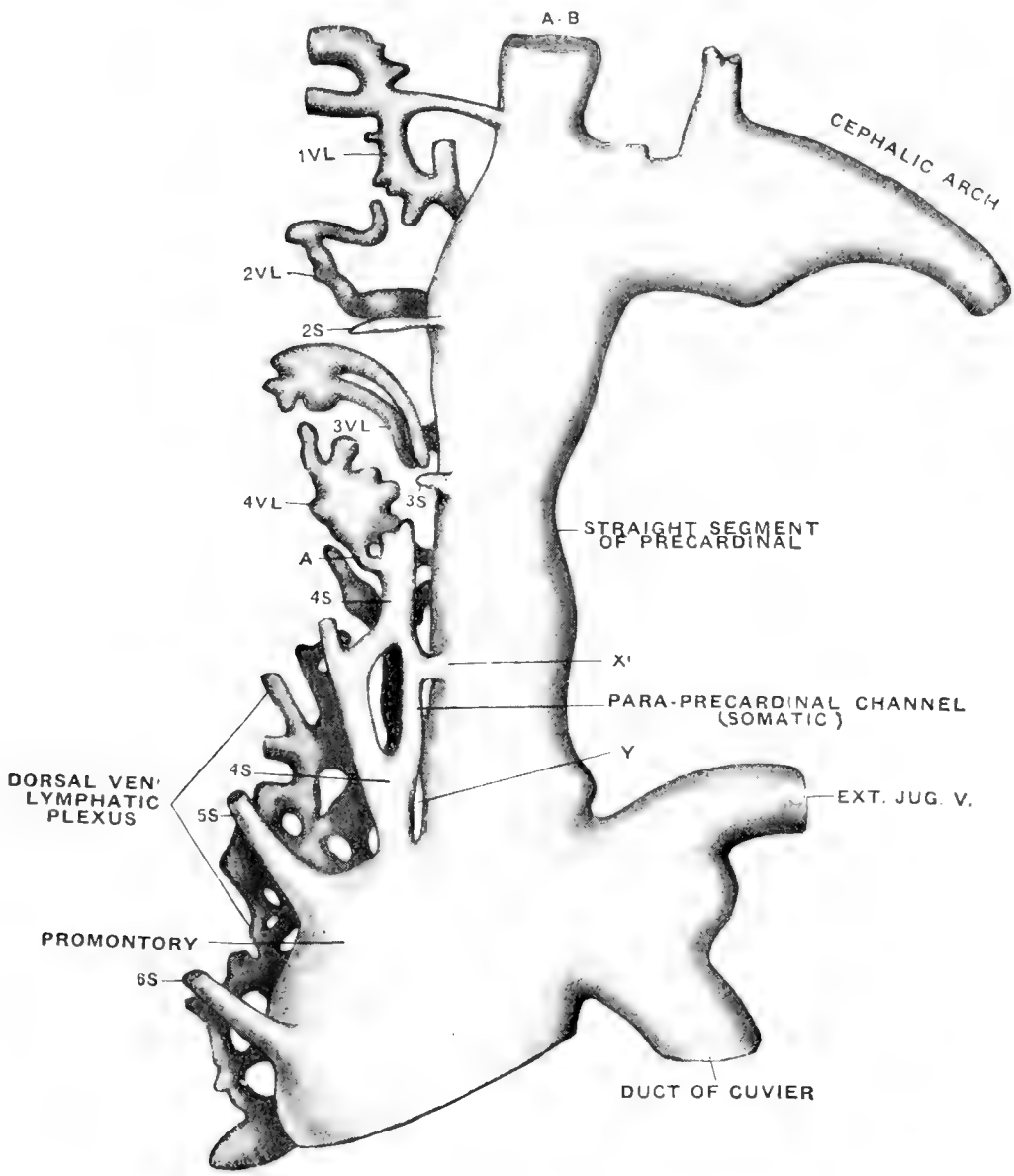
30

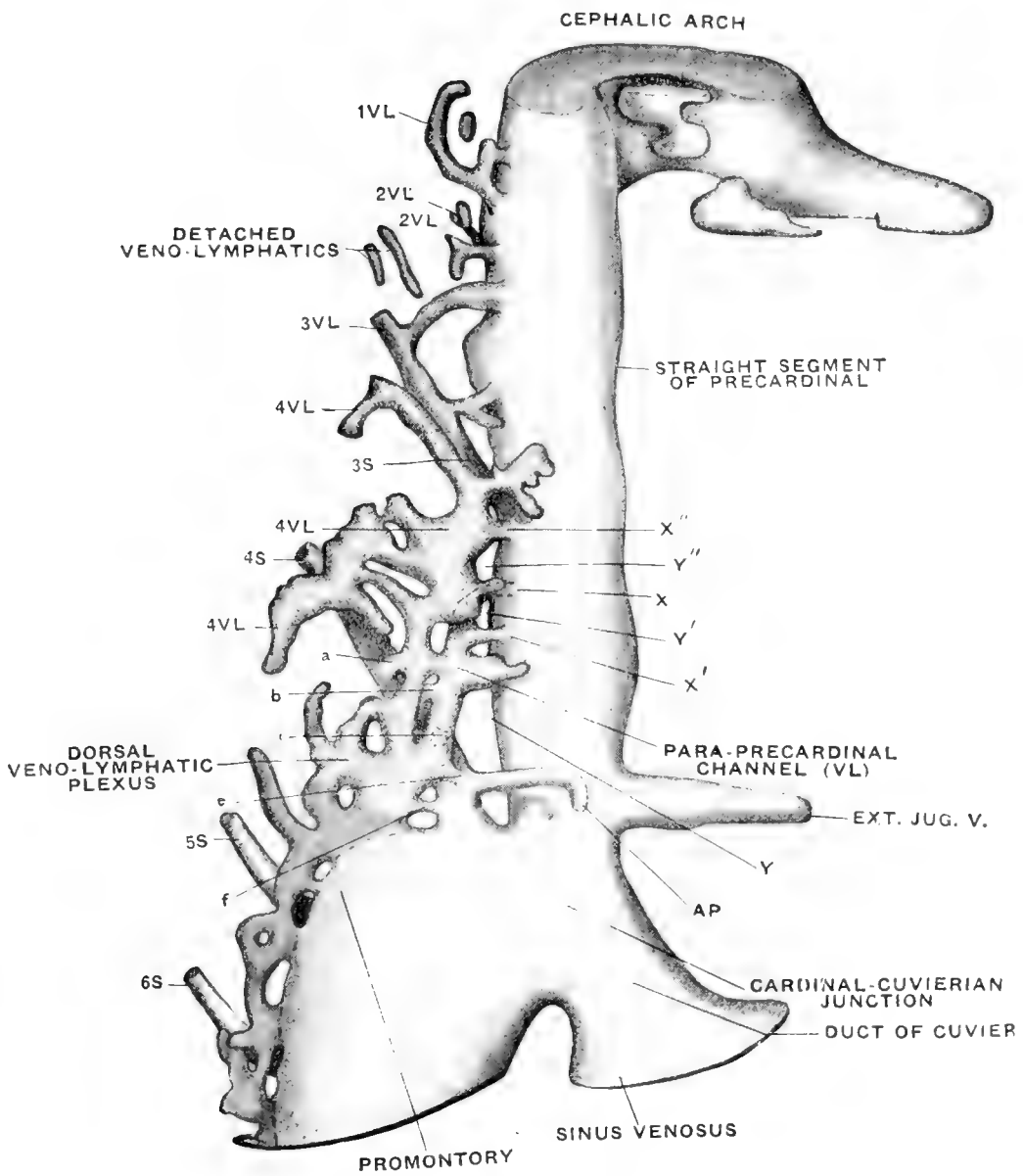

A-B
CEPHALIC ARCH
1
STRAIGHT SEGMENT
OF PRECARDINAL
2
4S
3VL
4VL
X
DORSAL VENO
LYMPHATIC
PLEXUS
PARA-PRECARDINAL
CHANNEL
Y
5S
EXT. JUG. V.
PROMONTORY
6S
DUCT OF CUVIER
POSTCARDINAL

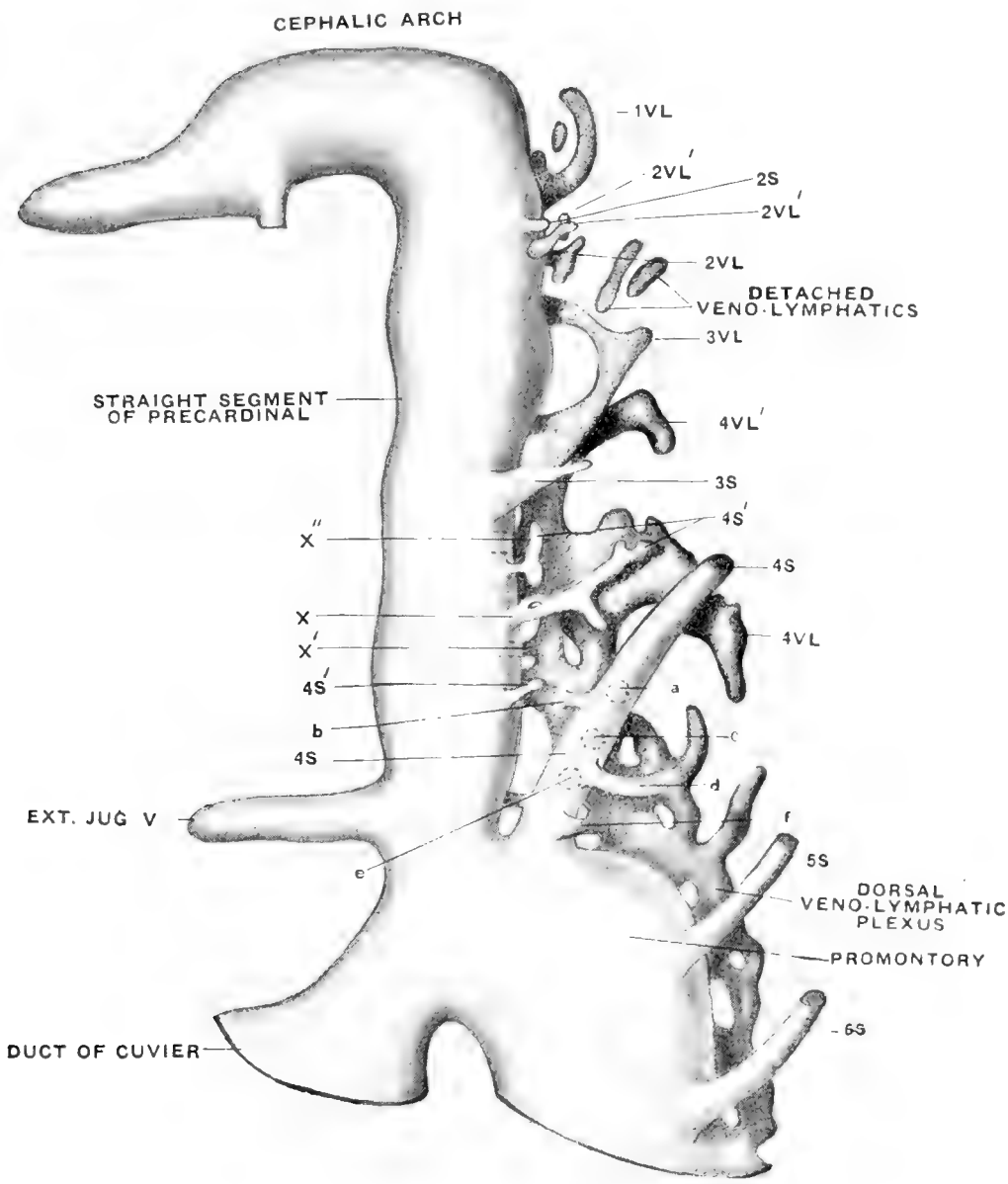
31

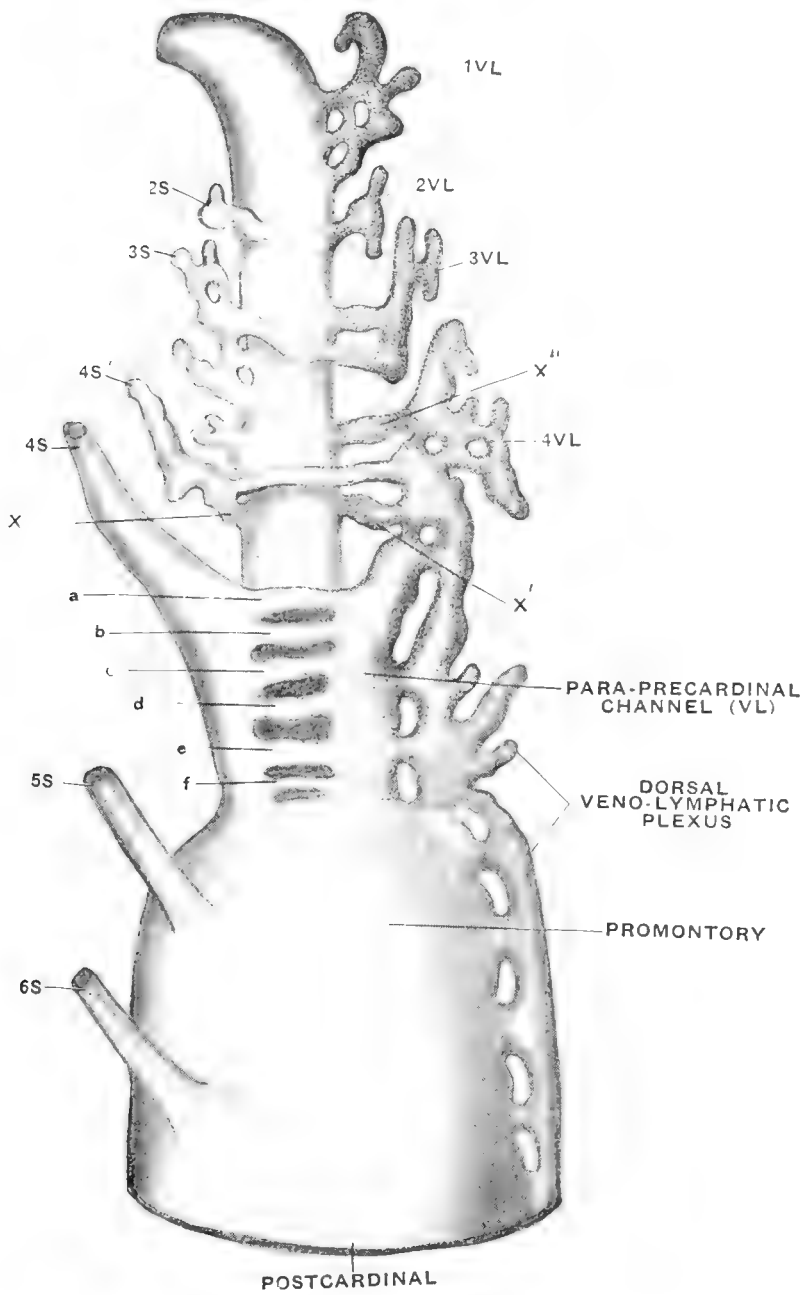

A-B
CEPHALIC ARCH
1
2
STRAIGHT SEGMENT OF PRECARDINAL
3
4
4VL
4VL
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS (PARA-PRECARDINAL CHANNEL)
Y
EXT. JUG. V.
Z
5S
CARDINAL-CUVIERIAN JUNCTION
PROMONTORY
DUCT OF CUVIER
6S
DORSAL VENO-LYMPHATIC PLEXUS
7S
POSTCARDINAL
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC


A-B
CEPHALIC ARCH
1VL
2VL
2S
3VL
STRAIGHT SEGMENT OF PRECARDINAL
3S
4VL
3VL'
A
X
4S
4VL'
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
PARA-PRECARDINAL CHANNEL (VL)
Y
AP
B
EXT. JUG. V.
5S
4S
DORSAL VENO-LYMPHATIC PLEXUS
PROMONTORY
6S
DUCT OF CUVIER

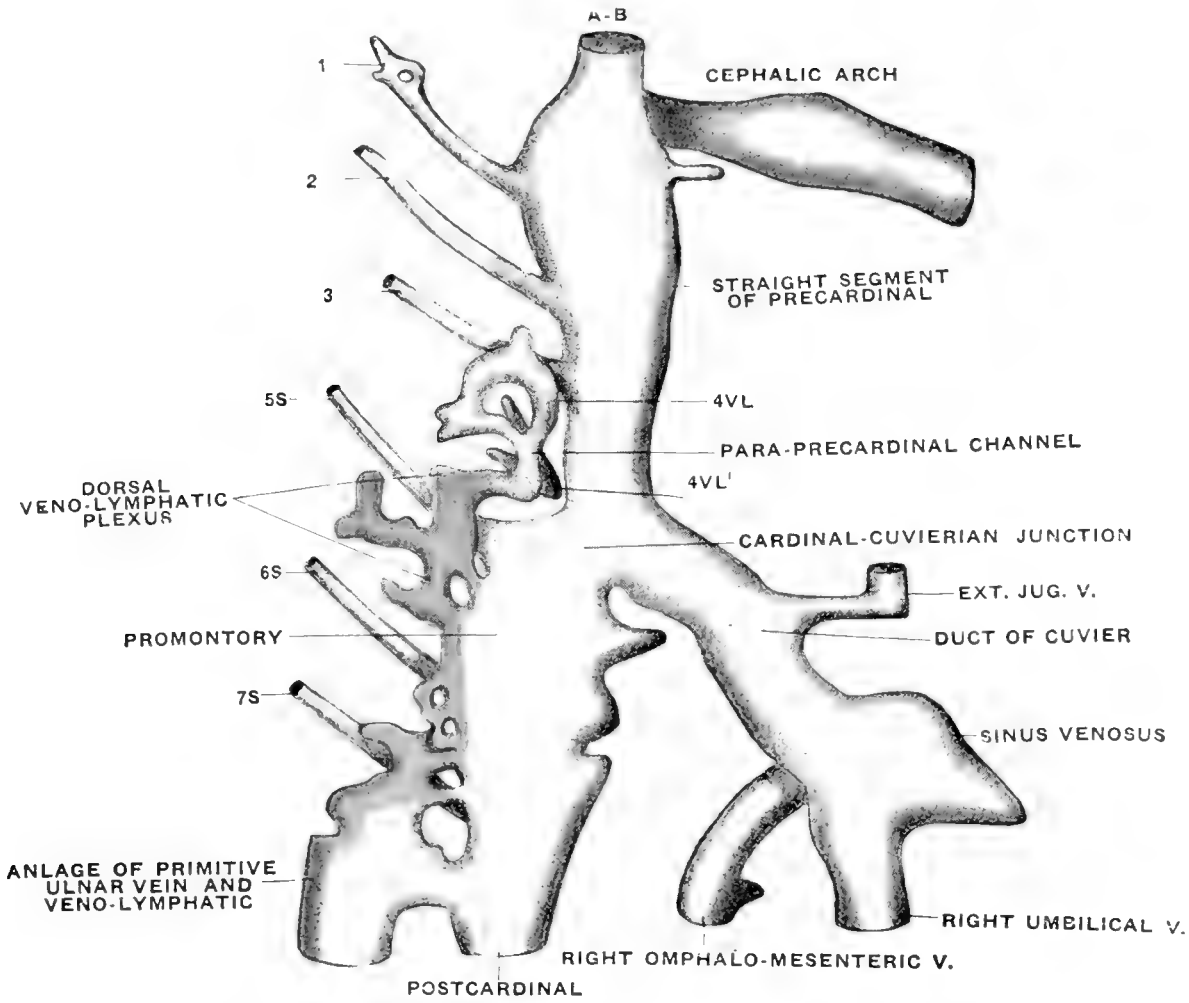

A-B
CEPHALIC ARCH
1VL
2VL
2S
3VL
4VL
3S
A
4S
STRAIGHT SEGMENT OF PRECARDINAL
X'
PARA-PRECARDINAL CHANNEL (SOMATIC)
Y
DORSAL VEN' LYMPHATIC PLEXUS
4S
5S
EXT. JUG. V.
PROMONTORY
6S
DUCT OF CUVIER


CEPHALIC ARCH
1VL
2VL
2VL
DETACHED
VENO-LYMPHATICS
3VL
4VL
3S
4VL
4S
4VL
a
b
c
DORSAL
VENO-LYMPHATIC
PLEXUS
e
5S
f
6S
STRAIGHT SEGMENT
OF PRECARDINAL
X''
Y''
X
Y'
X'
PARA-PRECARDINAL
CHANNEL (VL)
EXT. JUG. V.
Y
AP
CARDINAL-CUVIERIAN
JUNCTION
DUCT OF CUVIER
SINUS VENOSUS
PROMONTORY

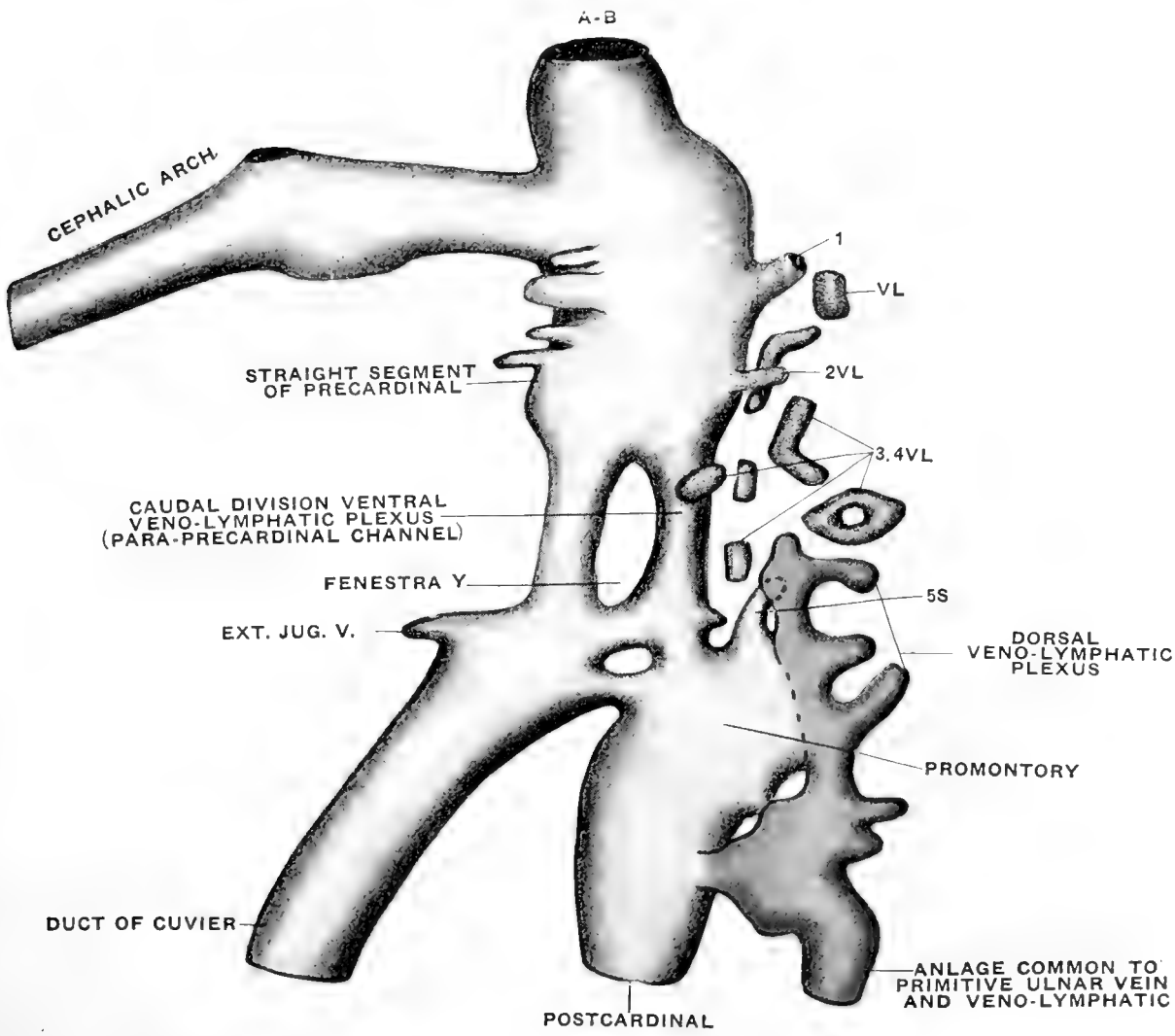

CEPHALIC ARCH
1VL
2VL'
2S
2VL'
2VL
DETACHED
VENO-LYMPHATICS
3VL
STRAIGHT SEGMENT
OF PRECARDINAL
4VL'
3S
4S'
X''
4S
X
4VL
X'
4S'
a
b
c
4S
d
EXT. JUG V
f
e
5S
DORSAL
VENO-LYMPHATIC
PLEXUS
PROMONTORY
6S
DUCT OF CUVIER


1VL
2S
2VL
3S
3VL
4S'
X''
4S
4VL
X
X'
a
b
c
d
e
f
5S
6S
PARA-PRECARDINAL CHANNEL (VL)
DORSAL VENO-LYMPHATIC PLEXUS
PROMONTORY
POSTCARDINAL

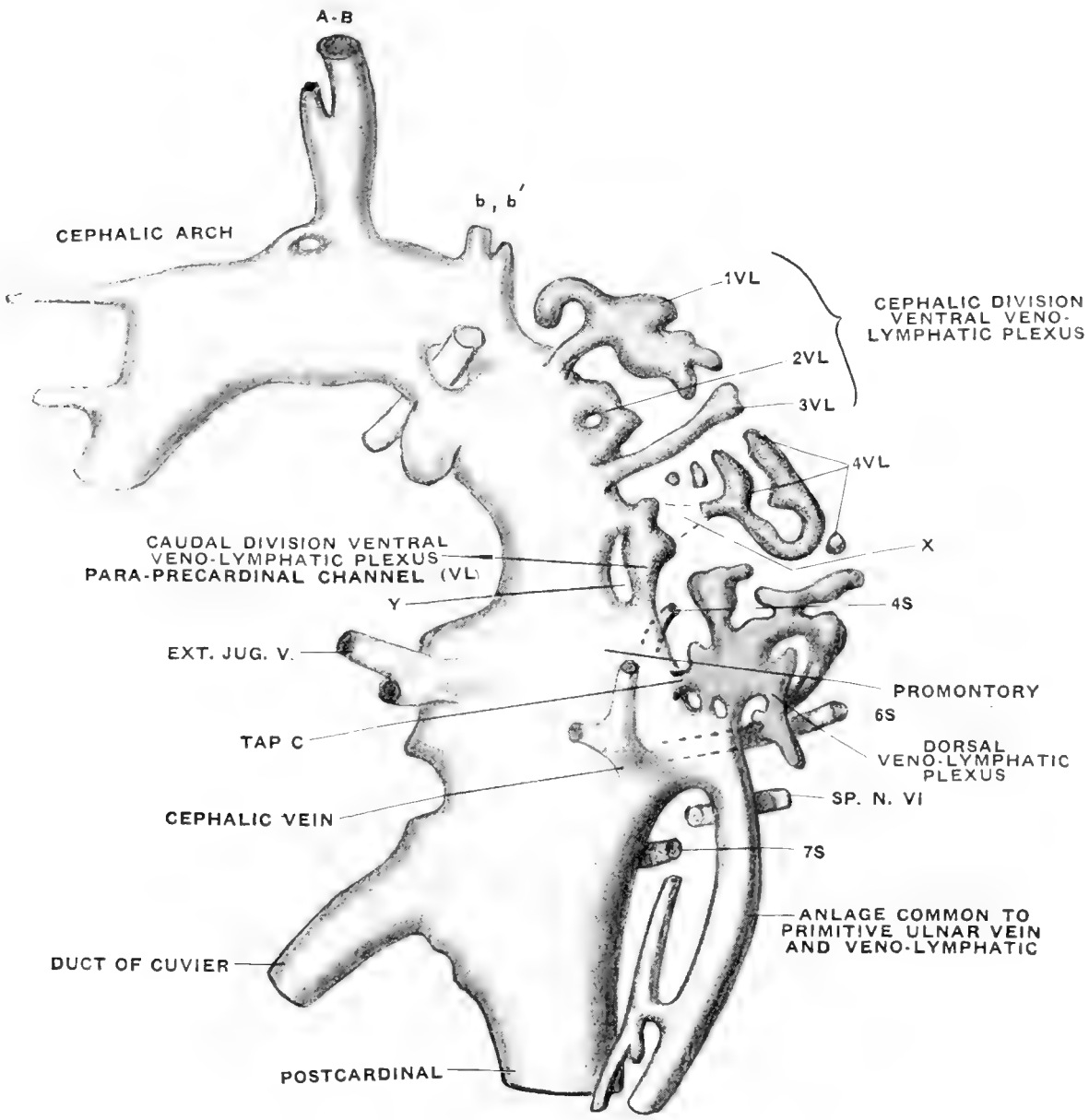
37

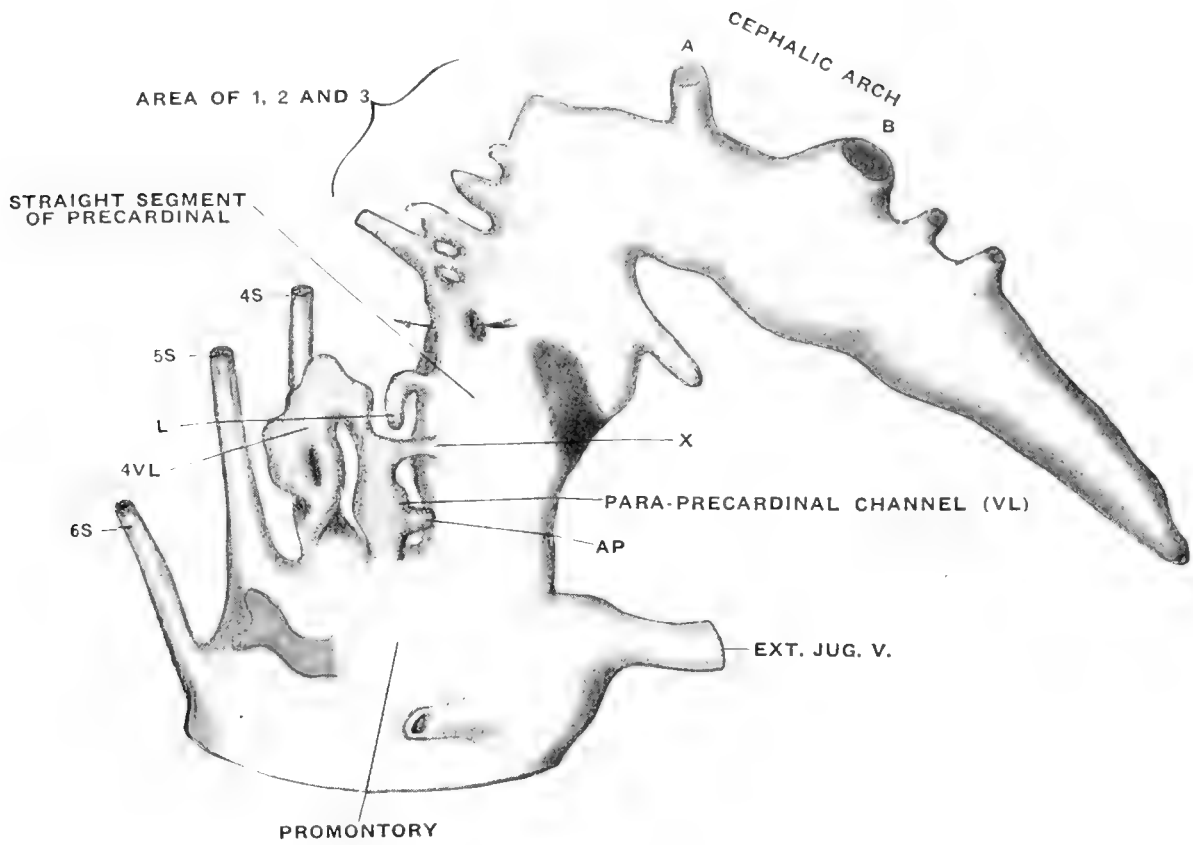

A-B
1
2
3
5S
6S
7S
CEPHALIC ARCH
STRAIGHT SEGMENT OF PRECARDINAL
4VL
PARA-PRECARDINAL CHANNEL
4VL'
DORSAL VENO-LYMPHATIC PLEXUS
CARDINAL-CUVIERIAN JUNCTION
EXT. JUG. V.
PROMONTORY
DUCT OF CUVIER
SINUS VENOSUS
ANLAGE OF PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
RIGHT UMBILICAL V.
RIGHT OMPHALO-MESENTERIC V.
POSTCARDINAL

38

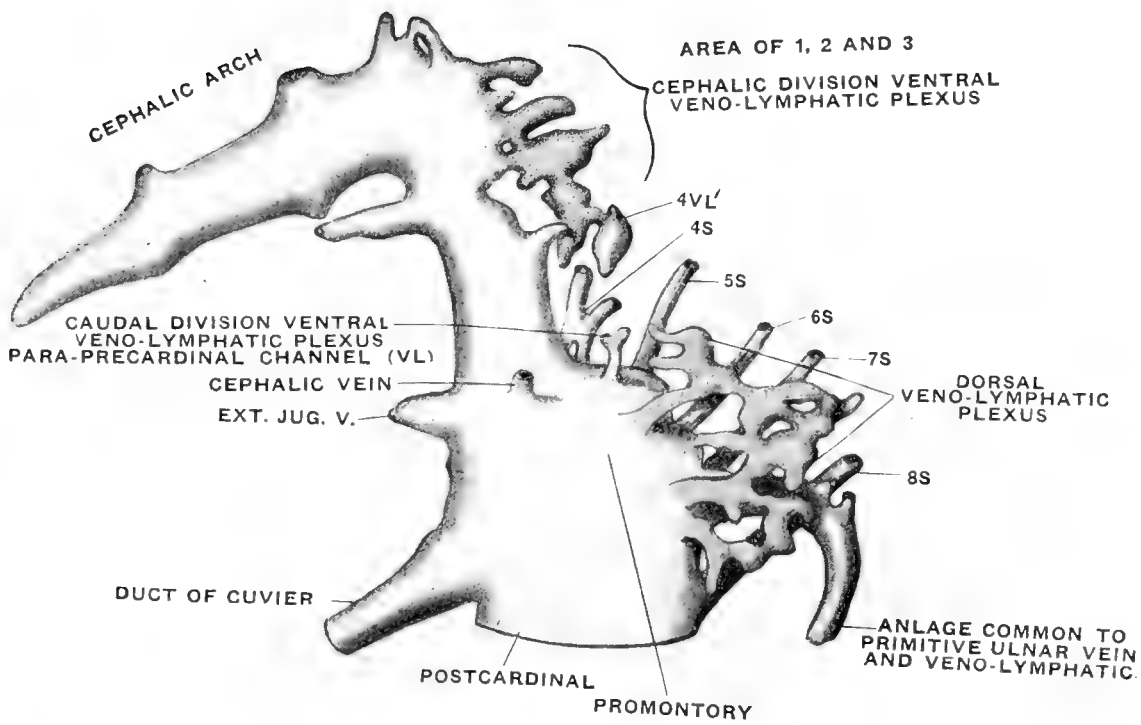

A-B
CEPHALIC ARCH
1
VL
2VL
STRAIGHT SEGMENT OF PRECARDINAL
3.4VL
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS (PARA-PRECARDINAL CHANNEL)
FENESTRA Y
5S
EXT. JUG. V.
DORSAL VENO-LYMPHATIC PLEXUS
PROMONTORY
DUCT OF CUVIER
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
POSTCARDINAL

39

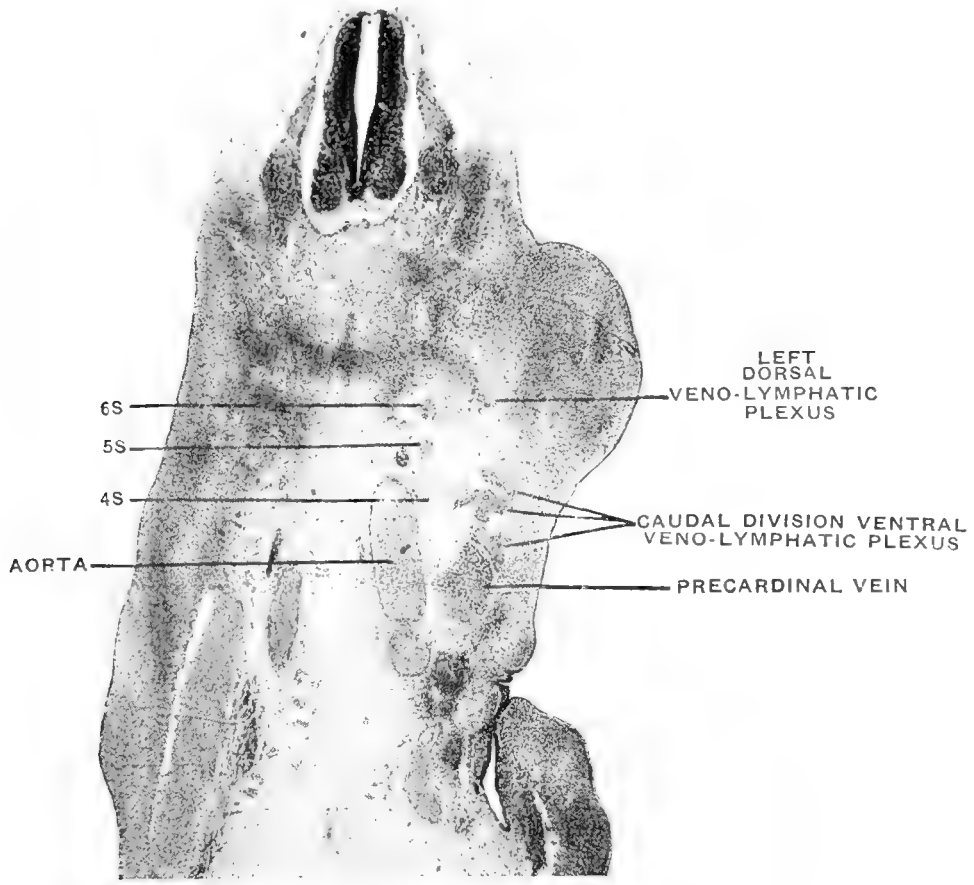

A-B
b, b′
CEPHALIC ARCH
1VL
2VL
3VL
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
4VL
X
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
PARA-PRECARDINAL CHANNEL (VL)
Y
4S
EXT. JUG. V.
PROMONTORY
6S
TAP C
DORSAL VENO-LYMPHATIC PLEXUS
SP. N. VI
CEPHALIC VEIN
7S
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
DUCT OF CUVIER
POSTCARDINAL


A
CEPHALIC ARCH
B
AREA OF 1, 2 AND 3
STRAIGHT SEGMENT OF PRECARDINAL
4S
5S
L
X
4VL
PARA-PRECARDINAL CHANNEL (VL)
6S
AP
EXT. JUG. V.
PROMONTORY

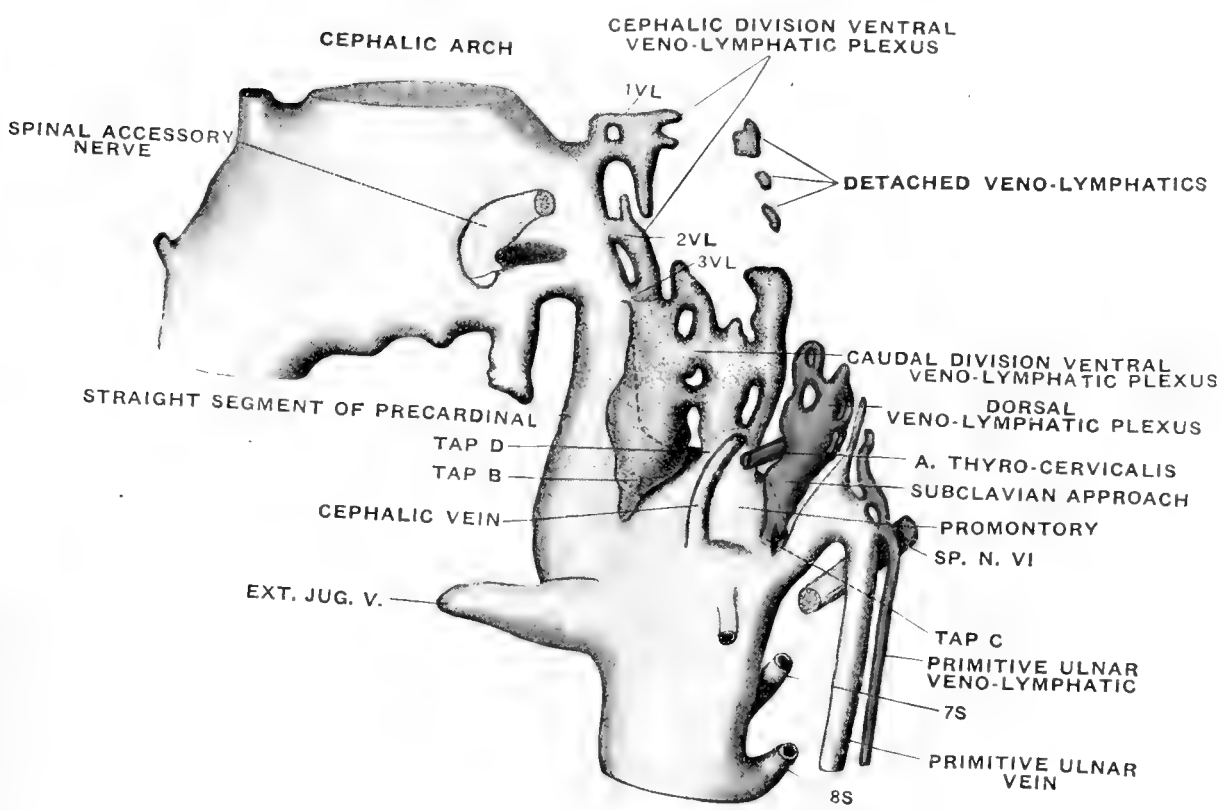
41


CEPHALIC ARCH
AREA OF 1, 2 AND 3
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
4VL'
4S
5S
6S
7S
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
PARA-PRECARDINAL CHANNEL (VL)
CEPHALIC VEIN
EXT. JUG. V.
DORSAL VENO-LYMPHATIC PLEXUS
8S
DUCT OF CUVIER
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
POSTCARDINAL
PROMONTORY

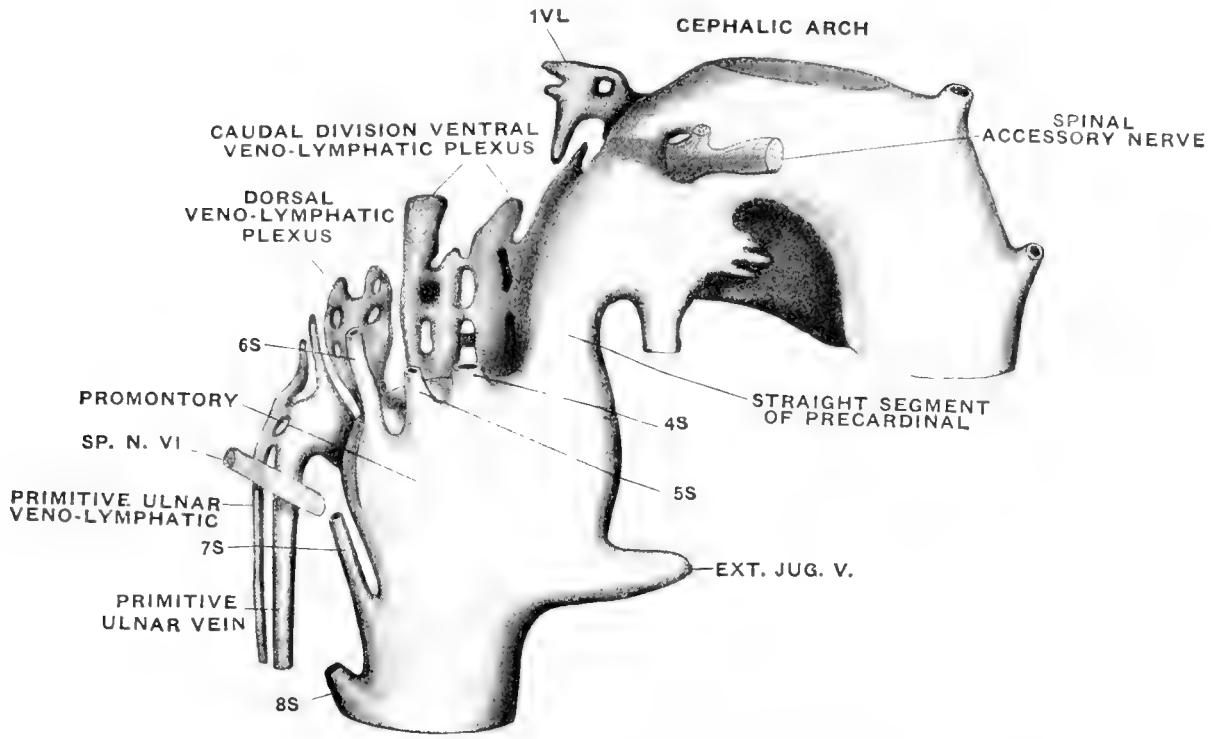
42


LEFT
DORSAL
VENO-LYMPHATIC
PLEXUS
6S
5S
4S
CAUDAL DIVISION VENTRAL
VENO-LYMPHATIC PLEXUS
AORTA
PRECARDINAL VEIN

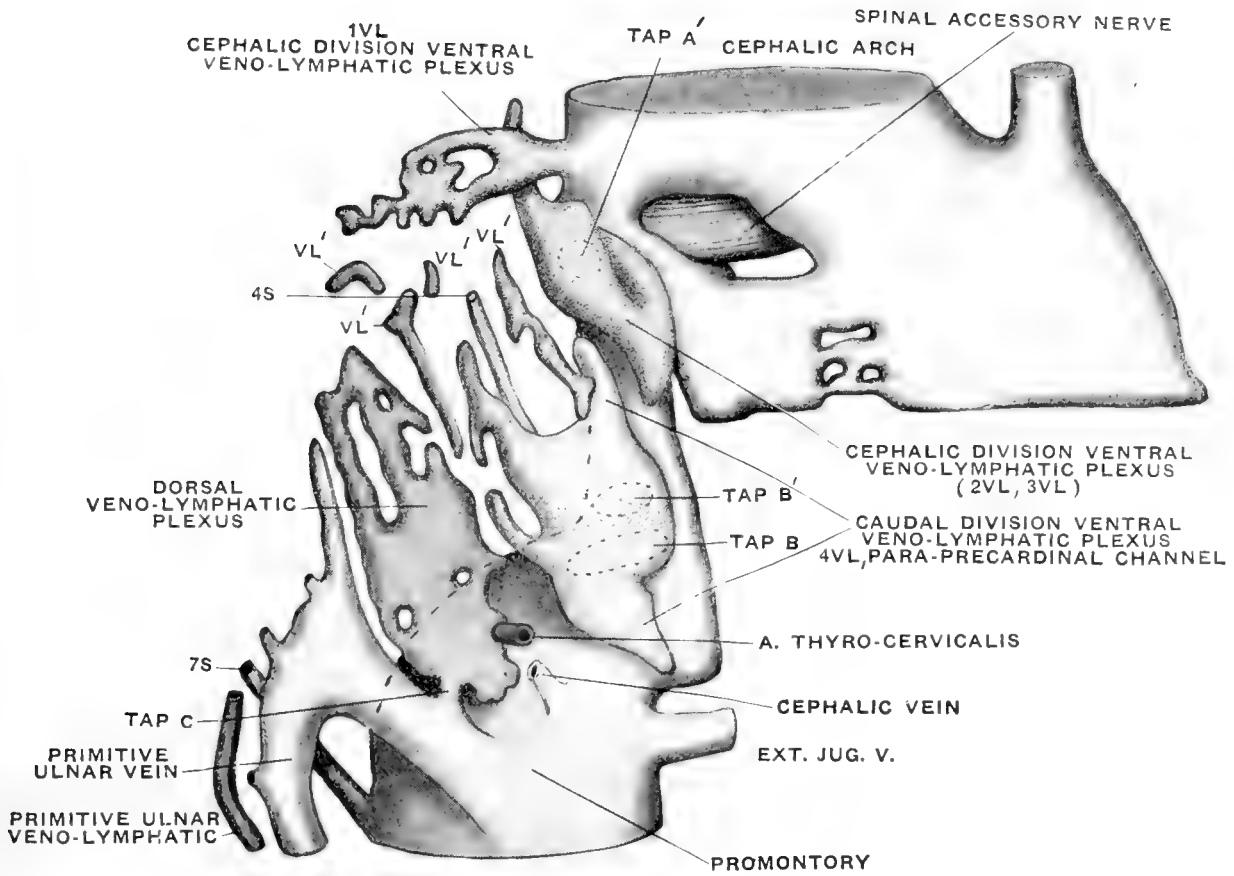
43


CEPHALIC ARCH
CEPHALIC DIVISION VENTRAL
VENO-LYMPHATIC PLEXUS
1VL
SPINAL ACCESSORY
NERVE
DETACHED VENO-LYMPHATICS
2VL
3VL
CAUDAL DIVISION VENTRAL
VENO-LYMPHATIC PLEXUS
STRAIGHT SEGMENT OF PRECARDINAL
DORSAL
VENO-LYMPHATIC PLEXUS
TAP D
TAP B
A. THYRO-CERVICALIS
SUBCLAVIAN APPROACH
CEPHALIC VEIN
PROMONTORY
SP. N. VI
EXT. JUG. V.
TAP C
PRIMITIVE ULNAR
VENO-LYMPHATIC
7S
PRIMITIVE ULNAR
VEIN
8S

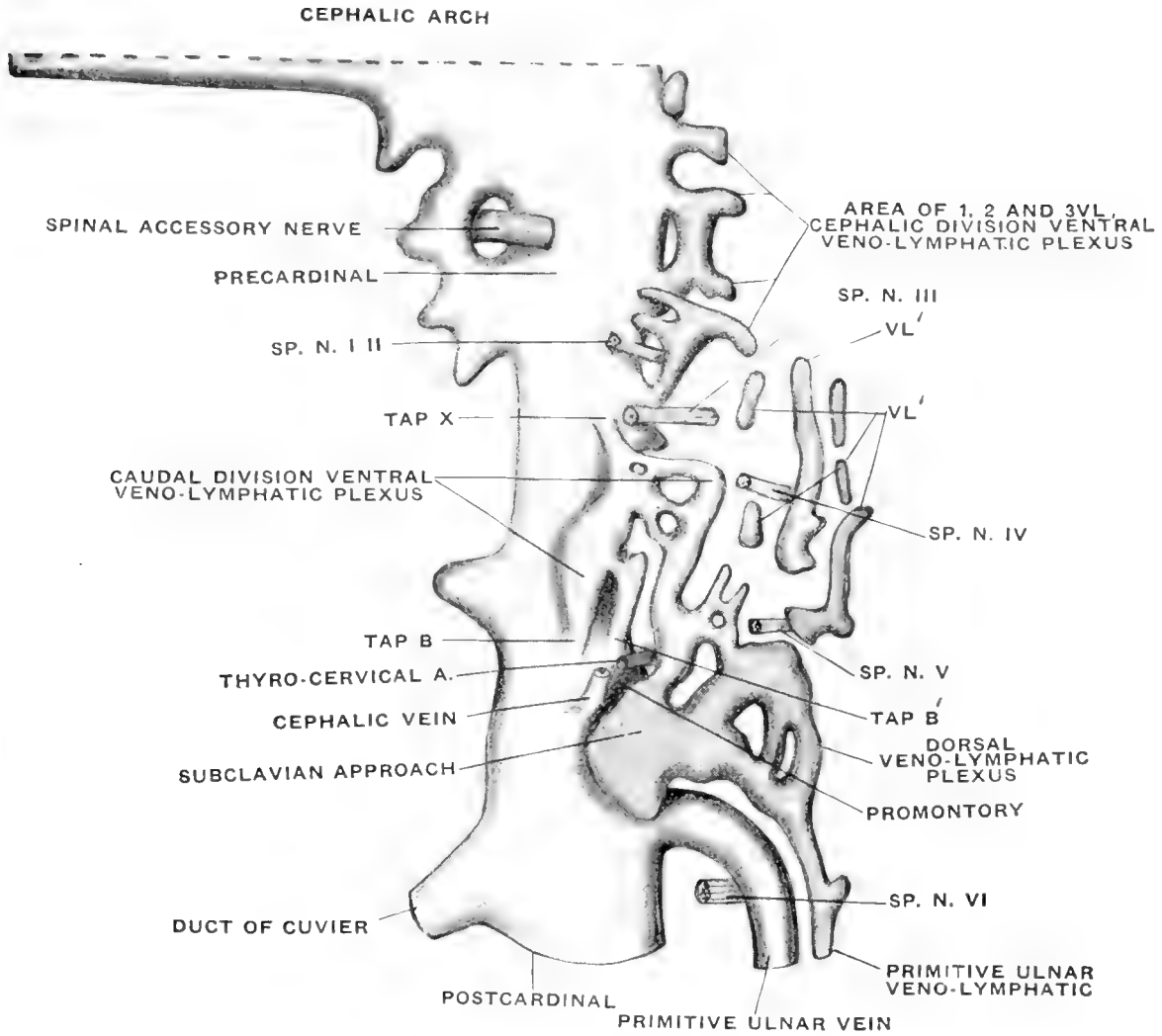
44


1VL
CEPHALIC ARCH
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SPINAL ACCESSORY NERVE
DORSAL VENO-LYMPHATIC PLEXUS
6S
PROMONTORY
SP. N. VI
PRIMITIVE ULNAR VENO-LYMPHATIC
7S
PRIMITIVE ULNAR VEIN
8S
4S
5S
STRAIGHT SEGMENT OF PRECARDINAL
EXT. JUG. V.

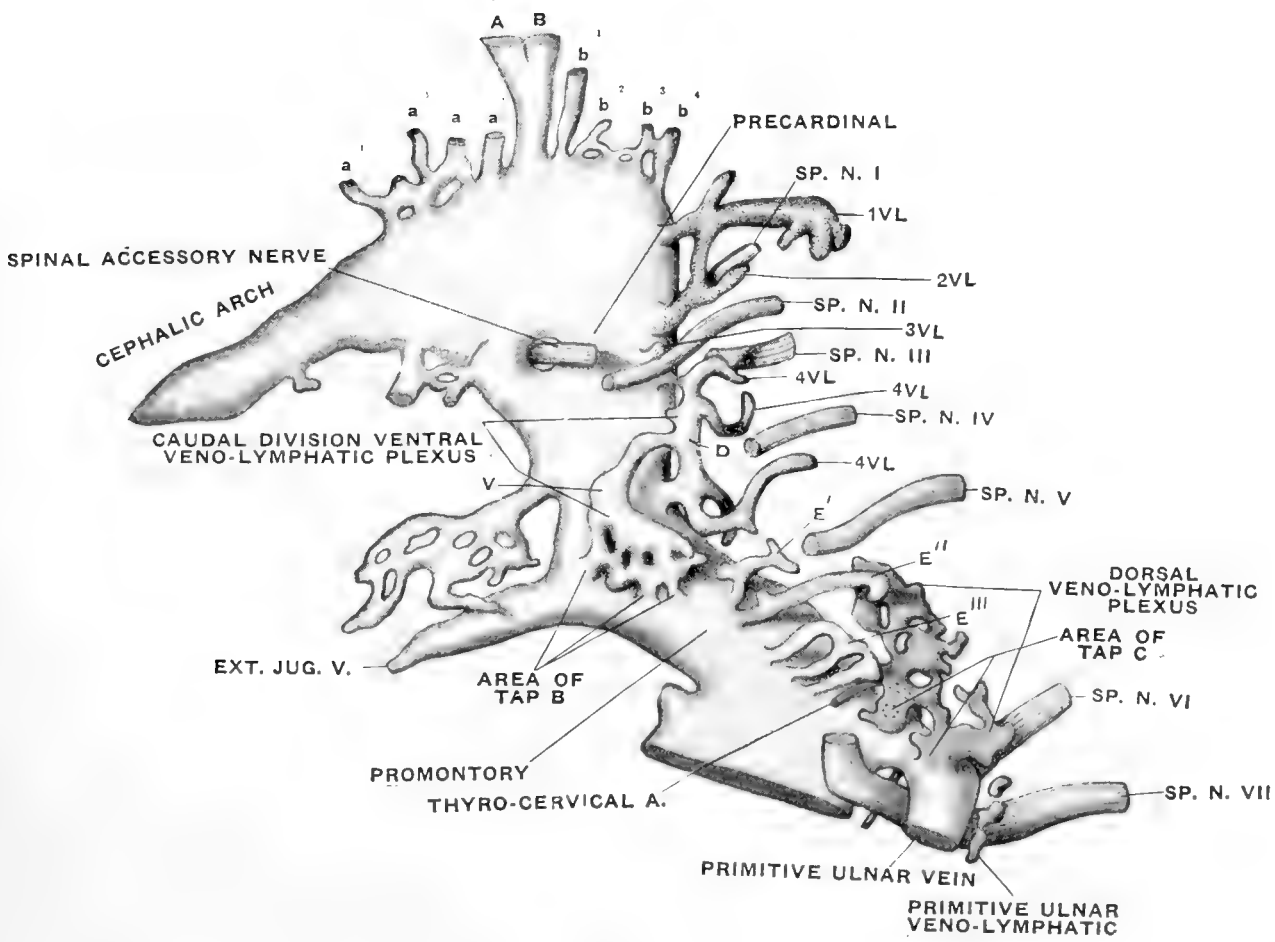
45


1VL
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
TAP A
CEPHALIC ARCH
SPINAL ACCESSORY NERVE
VL
VL
VL
4S
VL
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS (2VL, 3VL)
DORSAL VENO-LYMPHATIC PLEXUS
TAP B
TAP B
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS 4VL, PARA-PRECARDINAL CHANNEL
A. THYRO-CERVICALIS
7S
CEPHALIC VEIN
TAP C
EXT. JUG. V.
PRIMITIVE ULNAR VEIN
PRIMITIVE ULNAR VENO-LYMPHATIC
PROMONTORY

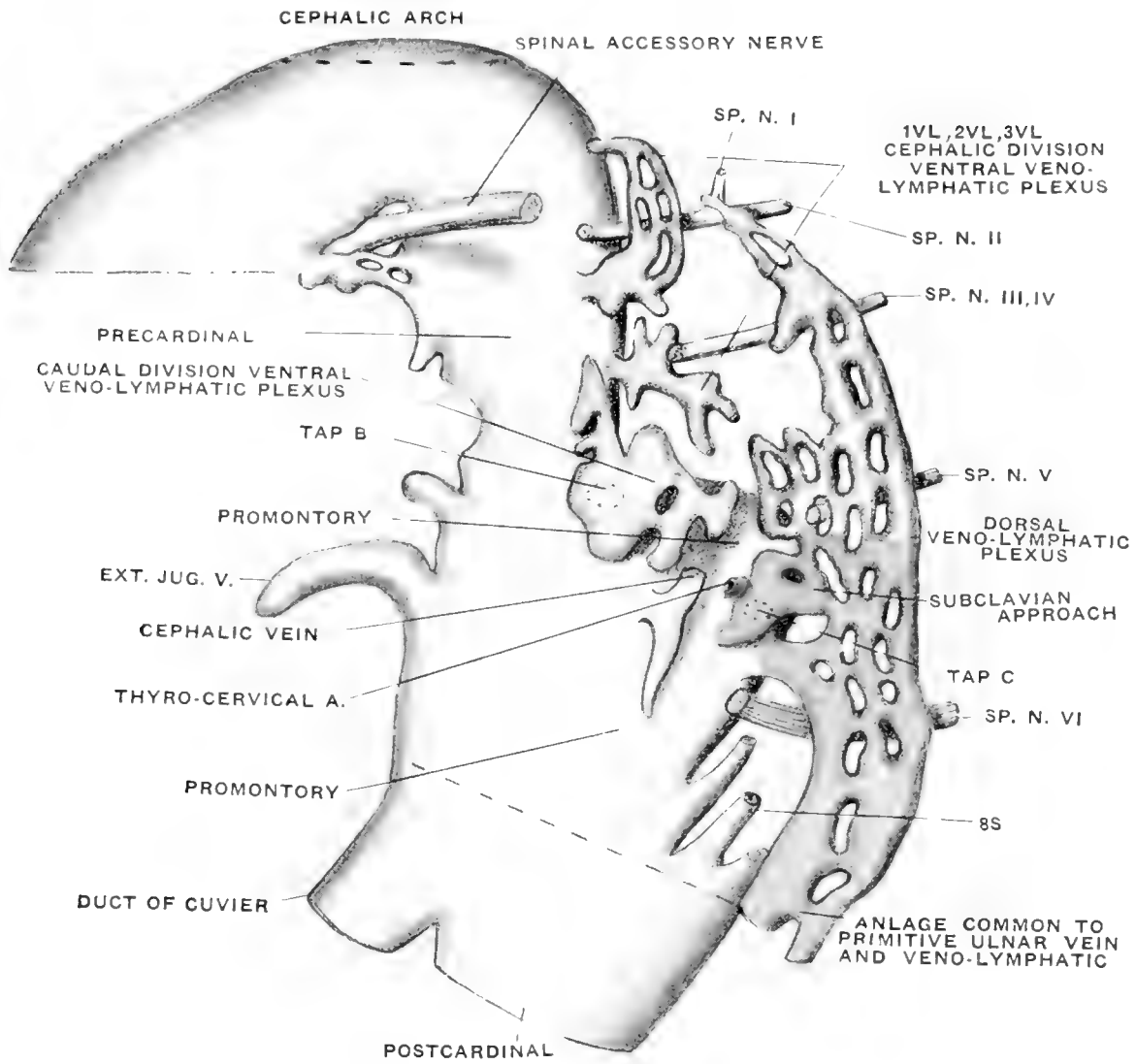
46


CEPHALIC ARCH
SPINAL ACCESSORY NERVE
PRECARDINAL
SP. N. I II
TAP X
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
TAP B
THYRO-CERVICAL A.
CEPHALIC VEIN
SUBCLAVIAN APPROACH
DUCT OF CUVIER
POSTCARDINAL
PRIMITIVE ULNAR VEIN
AREA OF 1, 2 AND 3VL, CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SP. N. III
VL'
VL'
SP. N. IV
SP. N. V
TAP B'
DORSAL VENO-LYMPHATIC PLEXUS
PROMONTORY
SP. N. VI
PRIMITIVE ULNAR VENO-LYMPHATIC

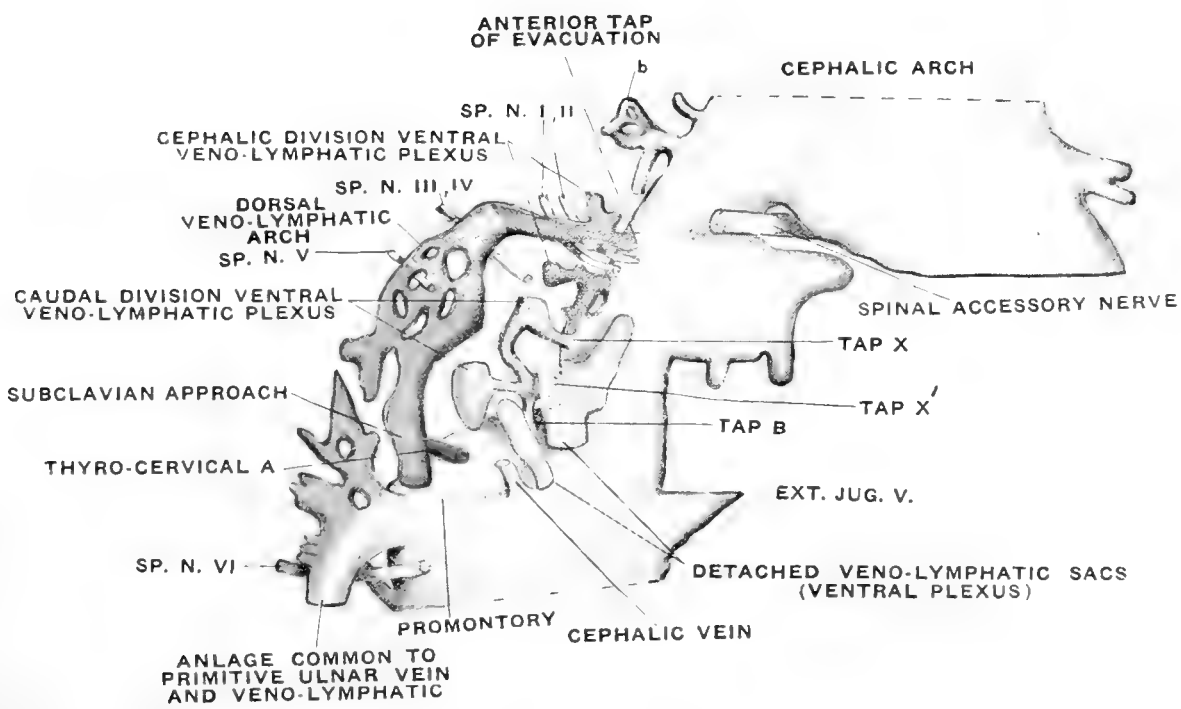
47


A B
b
a a a
b b b
a
PRECARDINAL
SP. N. I
1VL
SPINAL ACCESSORY NERVE
CEPHALIC ARCH
2VL
SP. N. II
3VL
SP. N. III
4VL
4VL
SP. N. IV
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
D
4VL
V
SP. N. V
E'
E''
DORSAL VENO-LYMPHATIC PLEXUS
E'''
AREA OF TAP C
EXT. JUG. V.
AREA OF TAP B
SP. N. VI
PROMONTORY
THYRO-CERVICAL A.
SP. N. VII
PRIMITIVE ULNAR VEIN
PRIMITIVE ULNAR VENO-LYMPHATIC

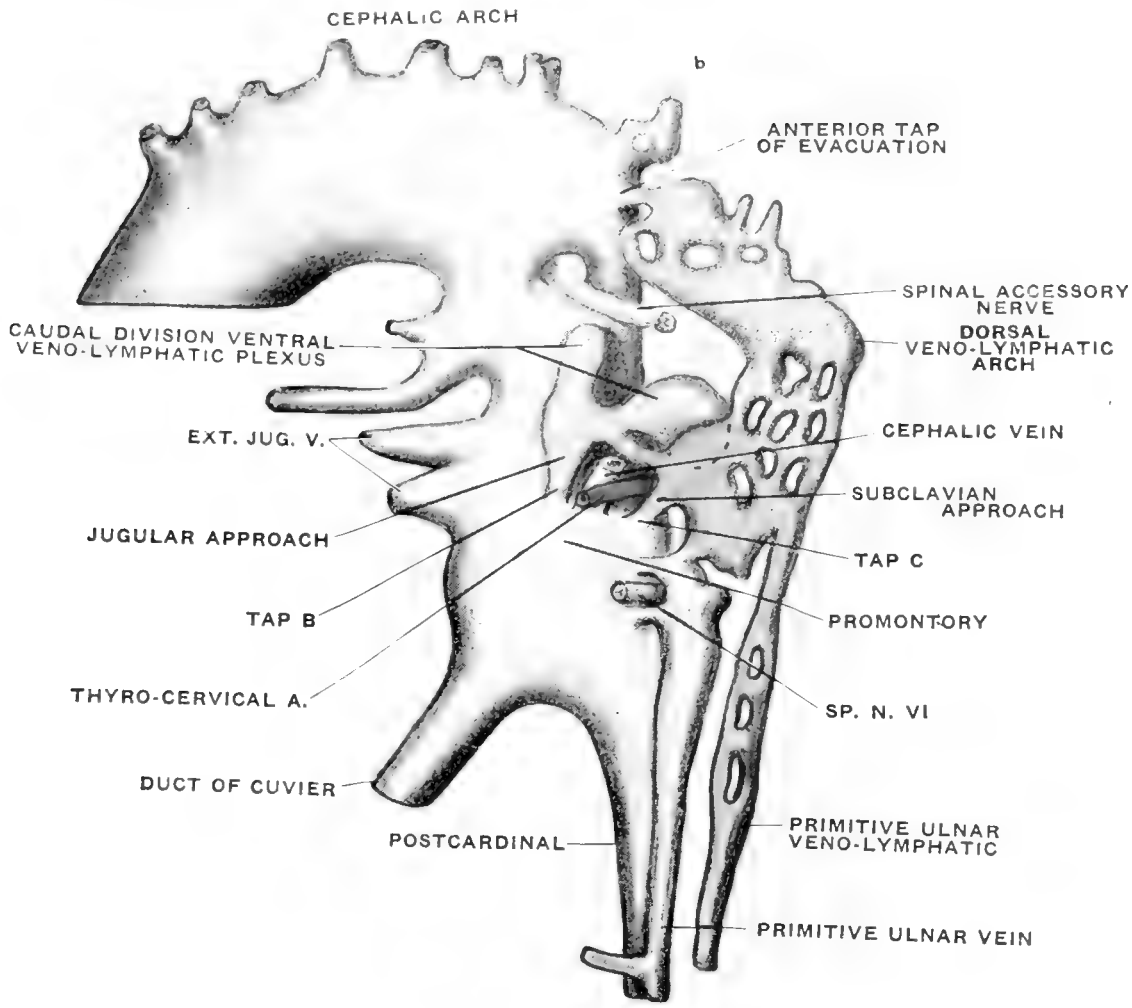
48


CEPHALIC ARCH
SPINAL ACCESSORY NERVE
SP. N. I
1VL, 2VL, 3VL CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SP. N. II
SP. N. III, IV
PRECARDINAL
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
TAP B
SP. N. V
PROMONTORY
DORSAL VENO-LYMPHATIC PLEXUS
EXT. JUG. V.
SUBCLAVIAN APPROACH
CEPHALIC VEIN
TAP C
THYRO-CERVICAL A.
SP. N. VI
PROMONTORY
8S
DUCT OF CUVIER
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC
POSTCARDINAL

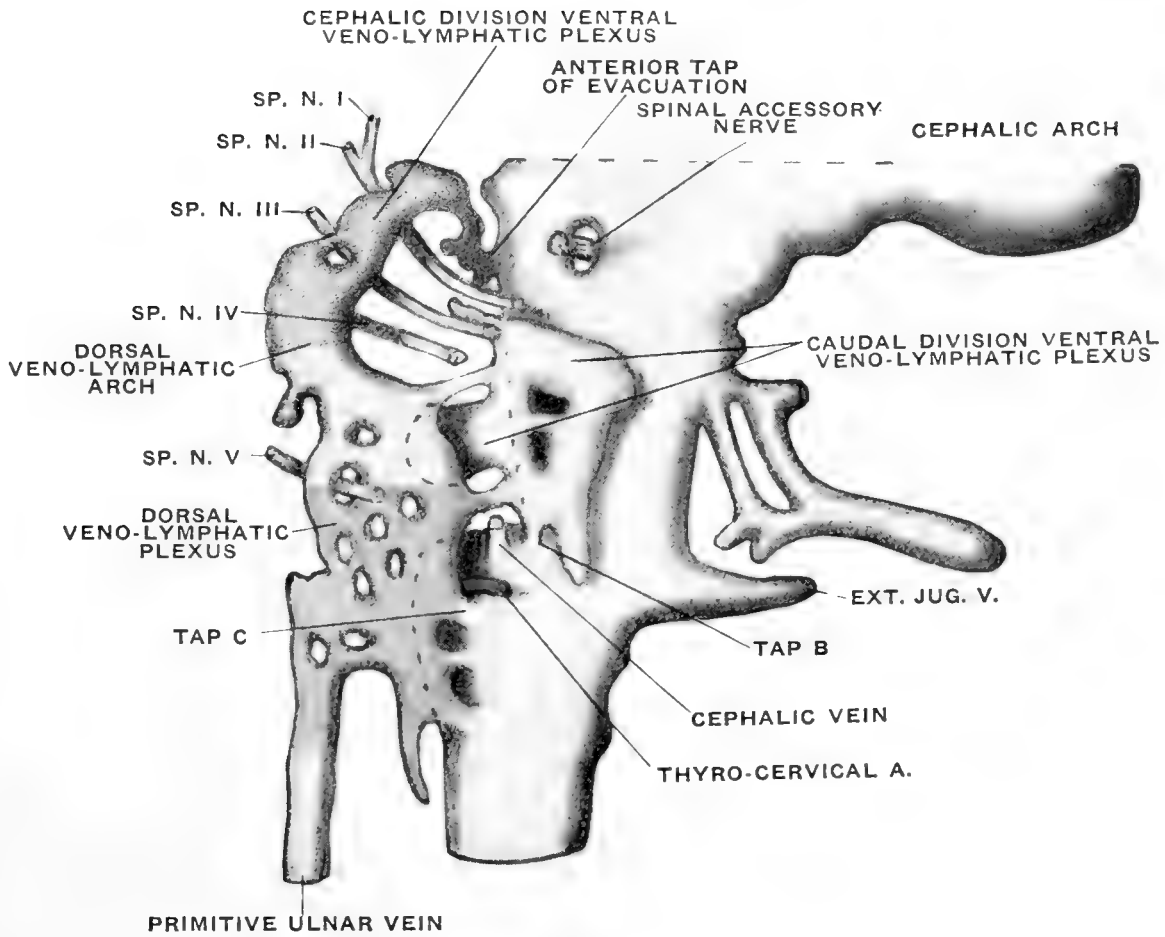
49


ANTERIOR TAP OF EVACUATION
b
CEPHALIC ARCH
SP. N. I, II
CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SP. N. III, IV
DORSAL VENO-LYMPHATIC ARCH
SP. N. V
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SPINAL ACCESSORY NERVE
TAP X
TAP X'
SUBCLAVIAN APPROACH
TAP B
THYRO-CERVICAL A
EXT. JUG. V.
SP. N. VI
DETACHED VENO-LYMPHATIC SACS (VENTRAL PLEXUS)
PROMONTORY
CEPHALIC VEIN
ANLAGE COMMON TO PRIMITIVE ULNAR VEIN AND VENO-LYMPHATIC

50


CEPHALIC ARCH
b
ANTERIOR TAP OF EVACUATION
SPINAL ACCESSORY NERVE
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
DORSAL VENO-LYMPHATIC ARCH
EXT. JUG. V.
CEPHALIC VEIN
SUBCLAVIAN APPROACH
JUGULAR APPROACH
TAP C
TAP B
PROMONTORY
THYRO-CERVICAL A.
SP. N. VI
DUCT OF CUVIER
POSTCARDINAL
PRIMITIVE ULNAR VENO-LYMPHATIC
PRIMITIVE ULNAR VEIN

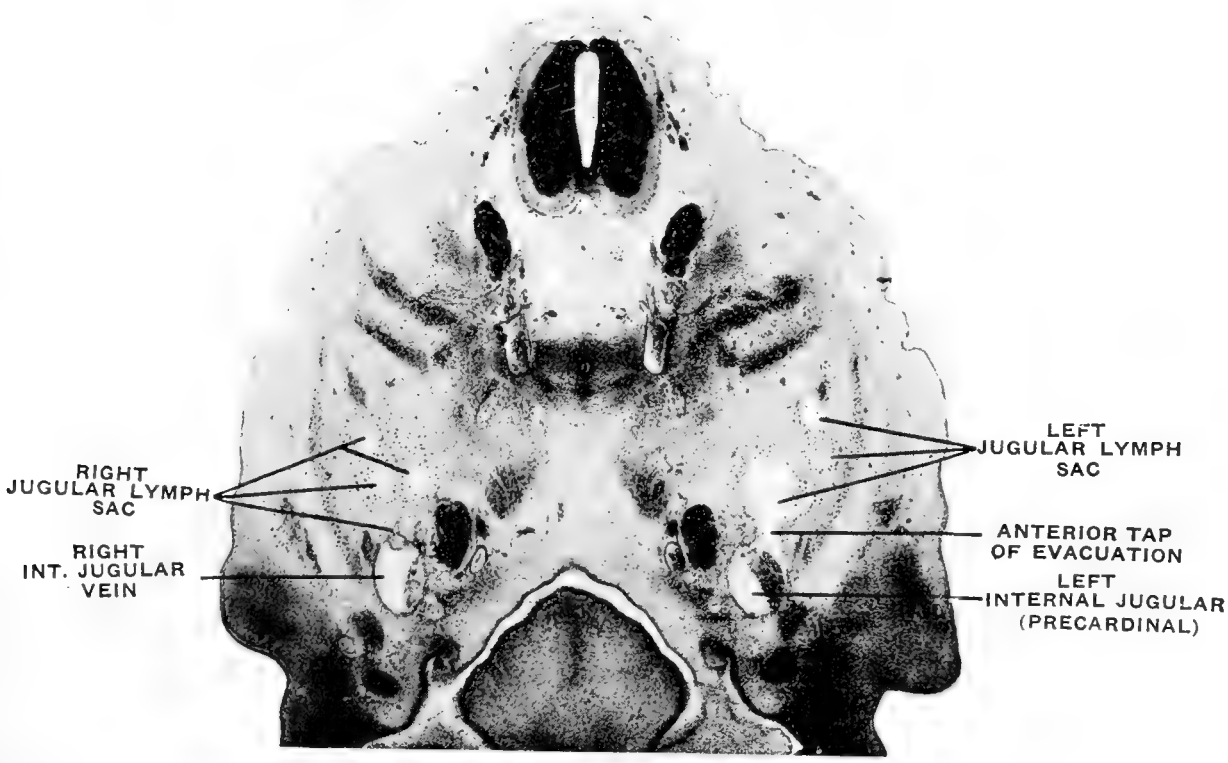
51


CEPHALIC DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
ANTERIOR TAP OF EVACUATION
SP. N. I
SP. N. II
SPINAL ACCESSORY NERVE
CEPHALIC ARCH
SP. N. III
SP. N. IV
DORSAL VENO-LYMPHATIC ARCH
CAUDAL DIVISION VENTRAL VENO-LYMPHATIC PLEXUS
SP. N. V
DORSAL VENO-LYMPHATIC PLEXUS
EXT. JUG. V.
TAP C
TAP B
CEPHALIC VEIN
THYRO-CERVICAL A.
PRIMITIVE ULNAR VEIN

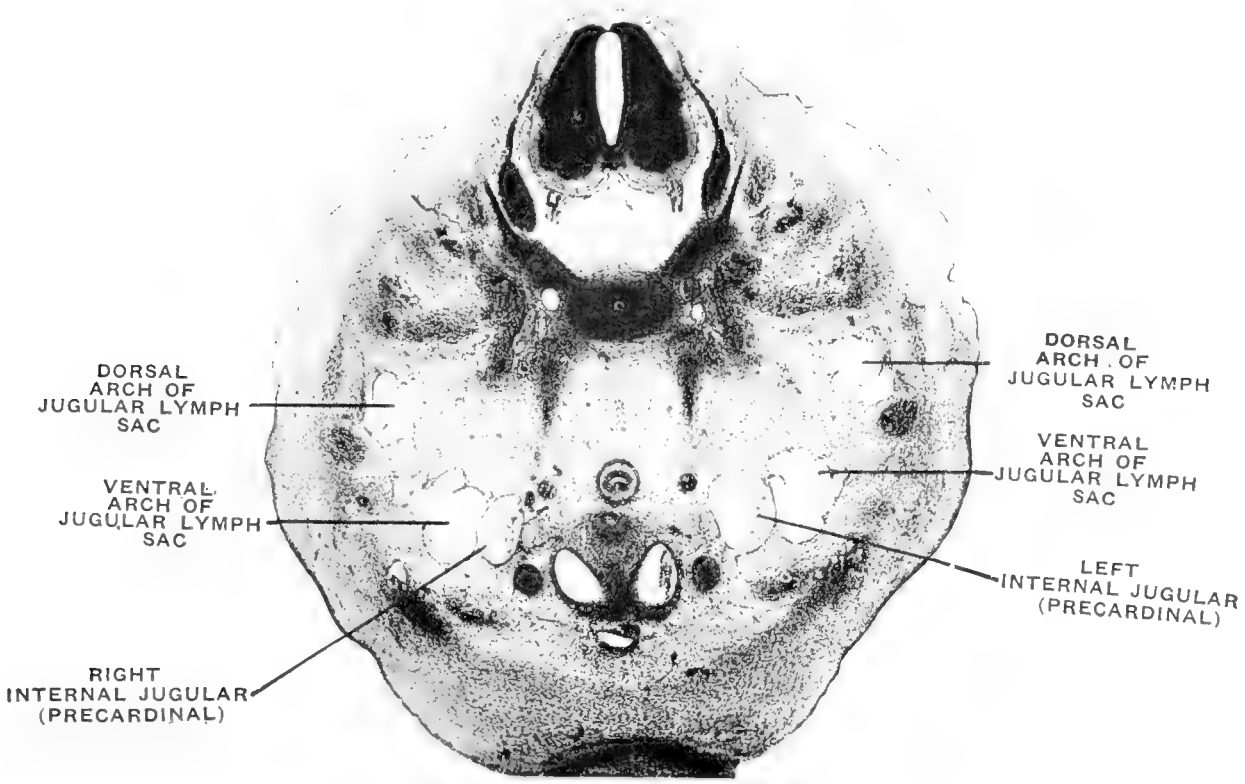
52


RIGHT
JUGULAR
LYMPH SAC
RIGHT
INT. JUGULAR
VEIN
LEFT
JUGULAR
LYMPH SAC
ANTERIOR TAP
OF EVACUATION
INTERNAL JUGULAR
(PRECARDINAL)

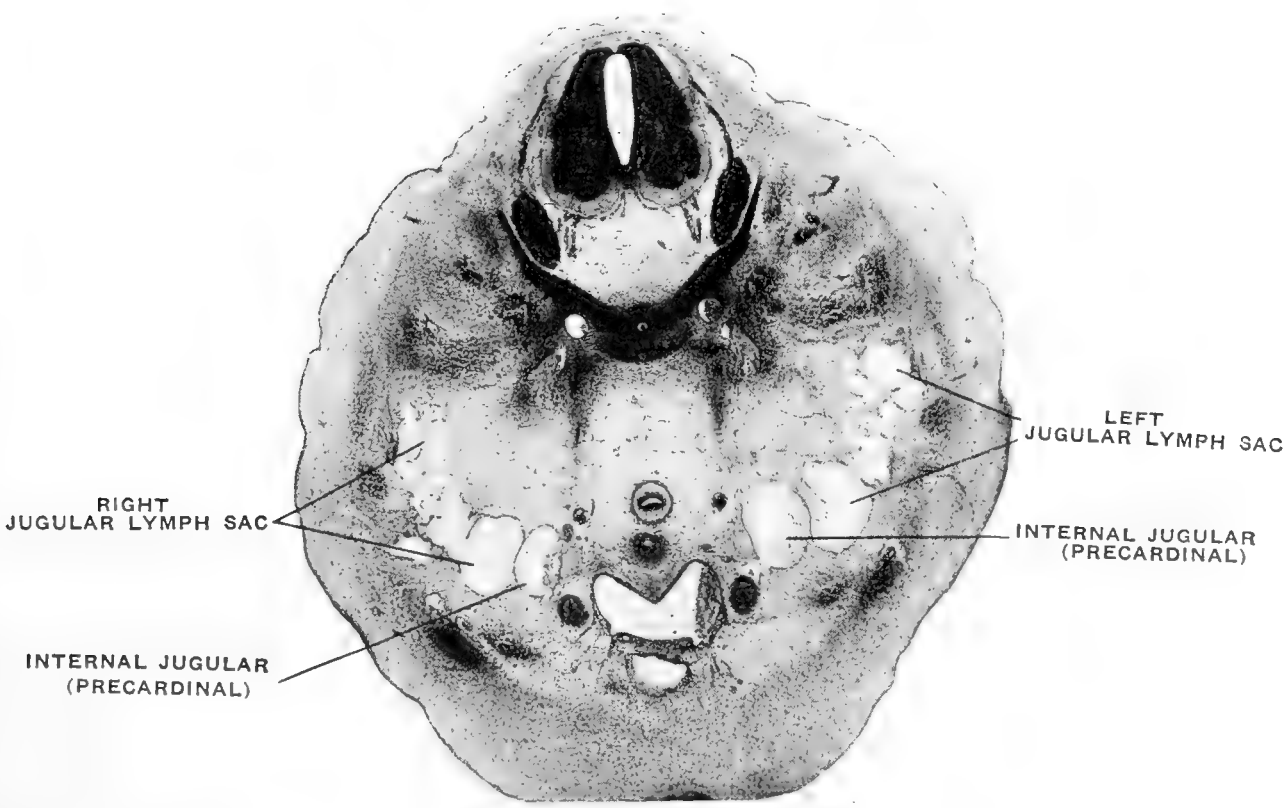
53


RIGHT
JUGULAR LYMPH
SAC
RIGHT
INT. JUGULAR
VEIN
LEFT
JUGULAR LYMPH
SAC
ANTERIOR TAP
OF EVACUATION
LEFT
INTERNAL JUGULAR
(PRECARDINAL)

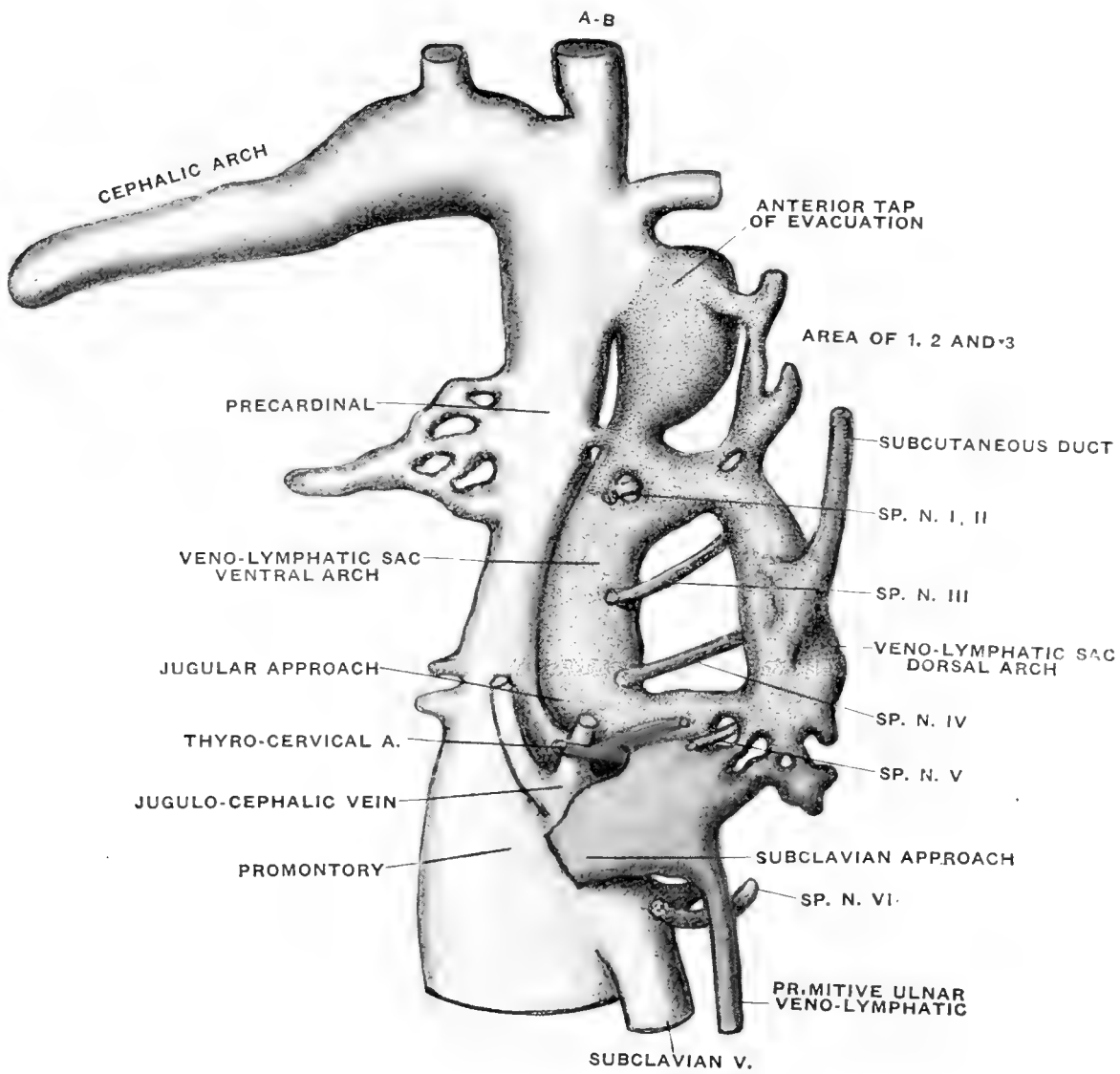
54

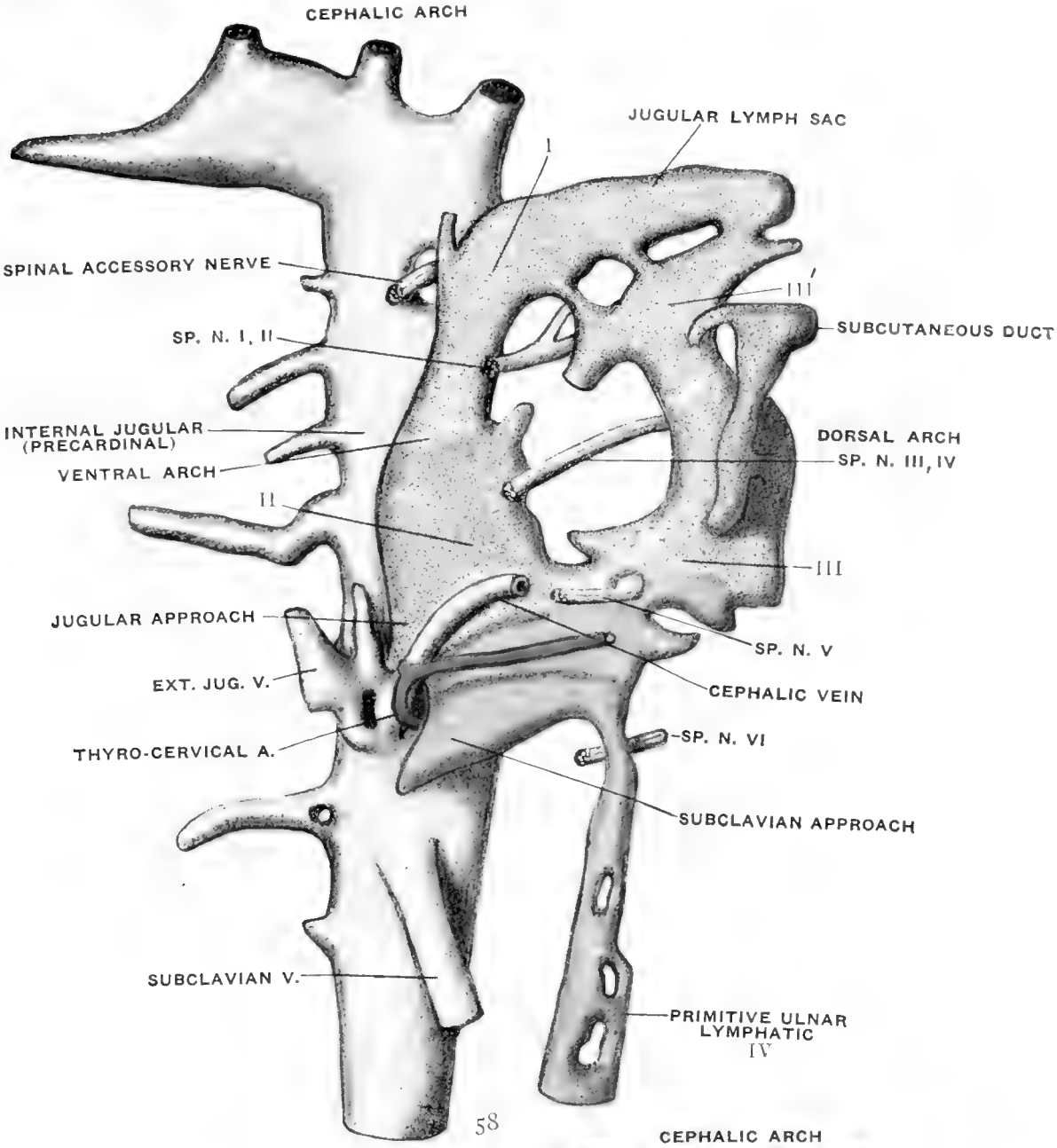

DORSAL ARCH OF JUGULAR LYMPH SAC
VENTRAL ARCH OF JUGULAR LYMPH SAC
RIGHT INTERNAL JUGULAR (PRECARDINAL)
DORSAL ARCH OF JUGULAR LYMPH SAC
VENTRAL ARCH OF JUGULAR LYMPH SAC
LEFT INTERNAL JUGULAR (PRECARDINAL)

55

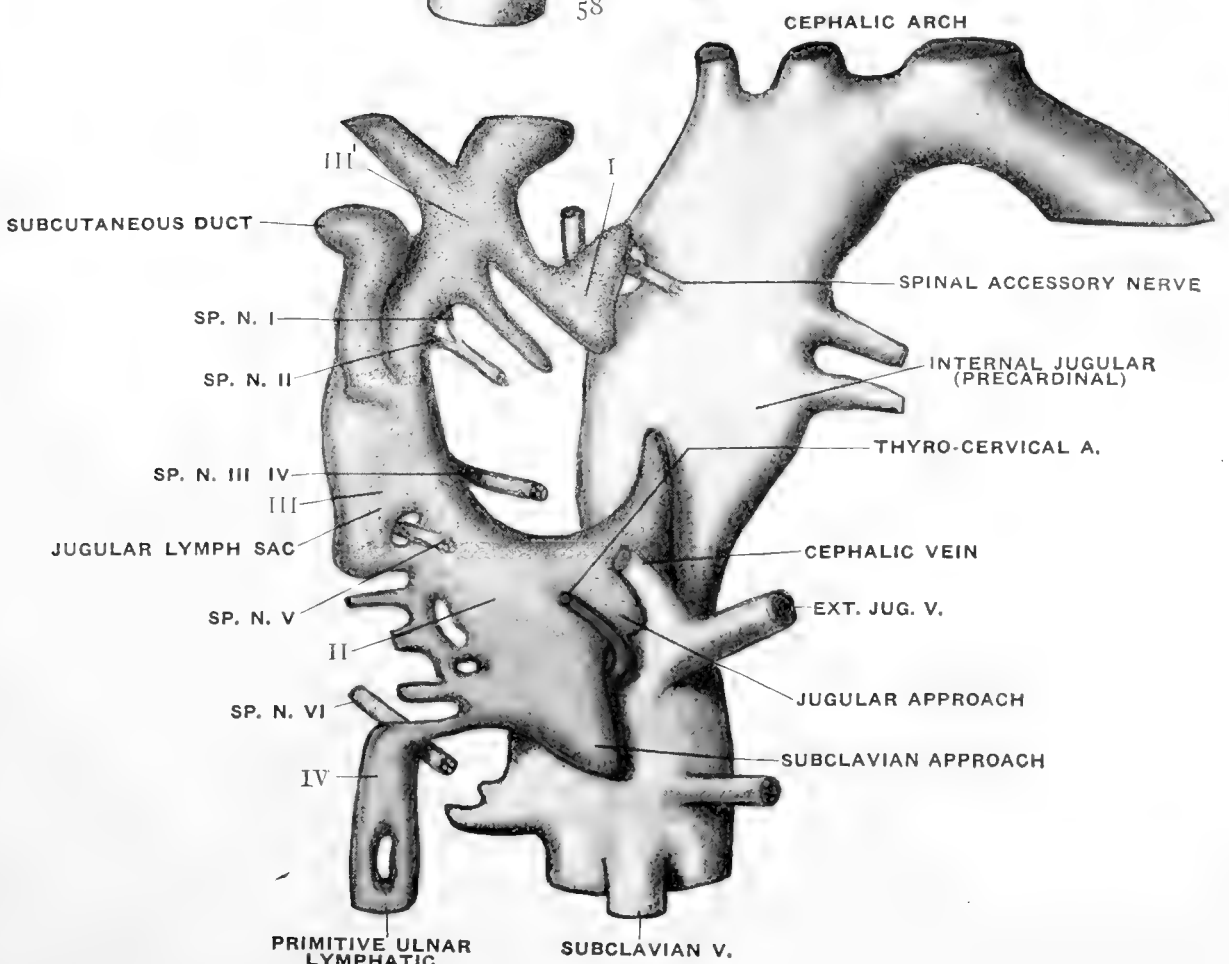

RIGHT JUGULAR LYMPH SAC
INTERNAL JUGULAR (PRECARDINAL)
LEFT JUGULAR LYMPH SAC
INTERNAL JUGULAR (PRECARDINAL)

56

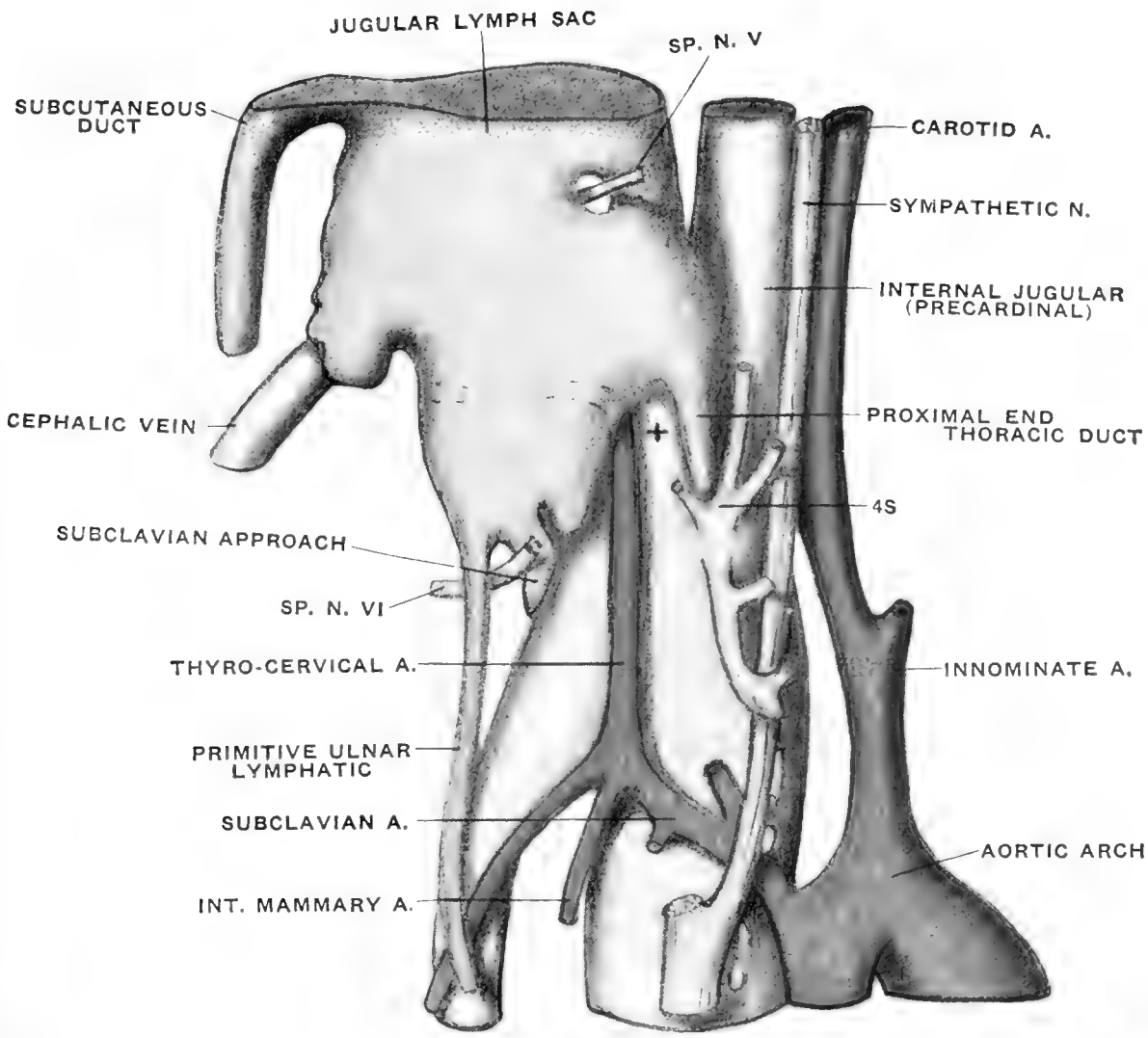

A-B
CEPHALIC ARCH
ANTERIOR TAP OF EVACUATION
AREA OF 1, 2 AND 3
PRECARDINAL
SUBCUTANEOUS DUCT
SP. N. I, II
VENO-LYMPHATIC SAC VENTRAL ARCH
SP. N. III
VENO-LYMPHATIC SAC DORSAL ARCH
JUGULAR APPROACH
SP. N. IV
THYRO-CERVICAL A.
SP. N. V
JUGULO-CEPHALIC VEIN
SUBCLAVIAN APPROACH
PROMONTORY
SP. N. VI
PRIMITIVE ULNAR VENO-LYMPHATIC
SUBCLAVIAN V.


CEPHALIC ARCH
JUGULAR LYMPH SAC
I
SPINAL ACCESSORY NERVE
III
SUBCUTANEOUS DUCT
SP. N. I, II
INTERNAL JUGULAR (PRECARDINAL)
DORSAL ARCH
VENTRAL ARCH
SP. N. III, IV
II
III
JUGULAR APPROACH
SP. N. V
EXT. JUG. V.
CEPHALIC VEIN
THYRO-CERVICAL A.
SP. N. VI
SUBCLAVIAN APPROACH
SUBCLAVIAN V.
PRIMITIVE ULNAR LYMPHATIC
IV

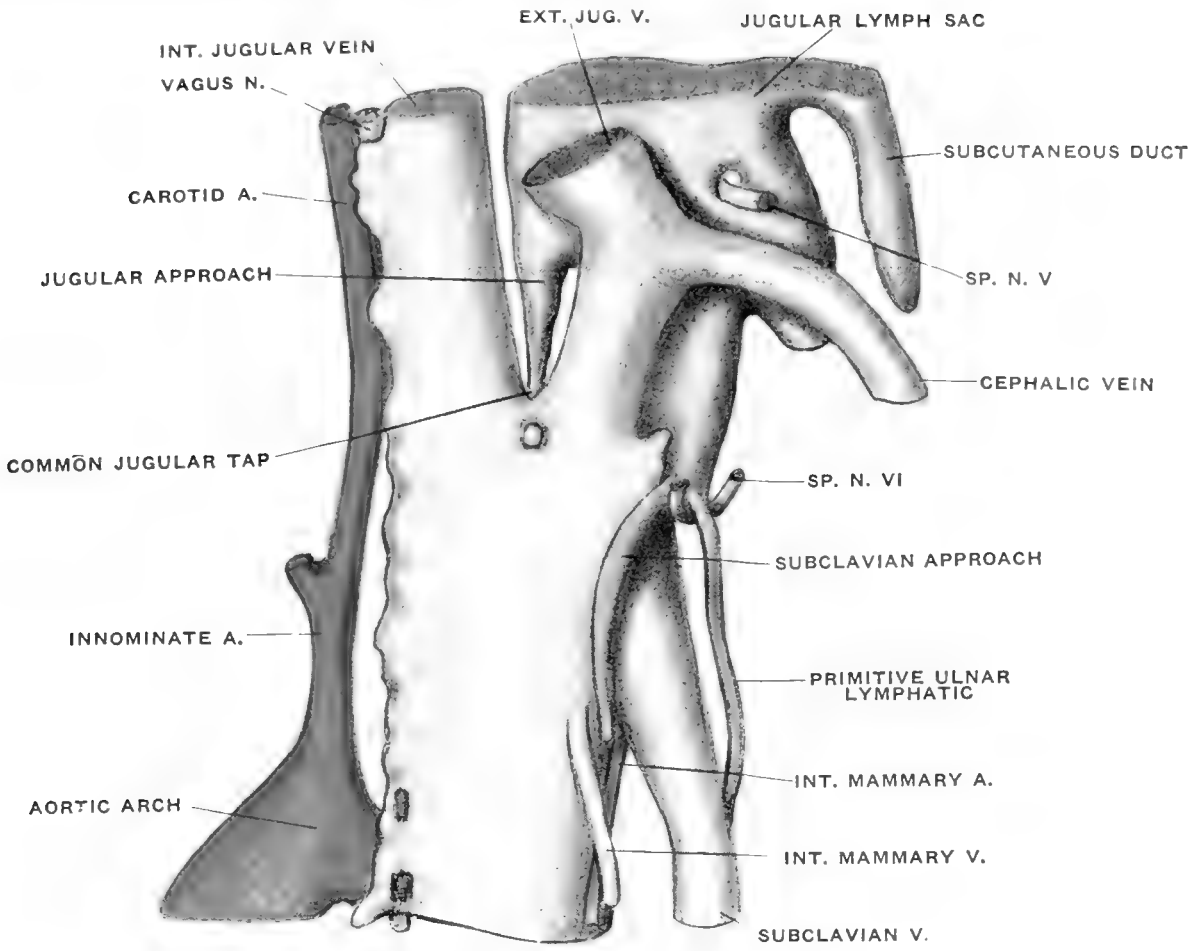
58


CEPHALIC ARCH
III
I
SUBCUTANEOUS DUCT
SPINAL ACCESSORY NERVE
SP. N. I
SP. N. II
INTERNAL JUGULAR (PRECARDINAL)
THYRO-CERVICAL A.
SP. N. III IV
III
JUGULAR LYMPH SAC
CEPHALIC VEIN
SP. N. V
EXT. JUG. V.
II
JUGULAR APPROACH
SP. N. VI
SUBCLAVIAN APPROACH
IV
PRIMITIVE ULNAR LYMPHATIC
SUBCLAVIAN V.

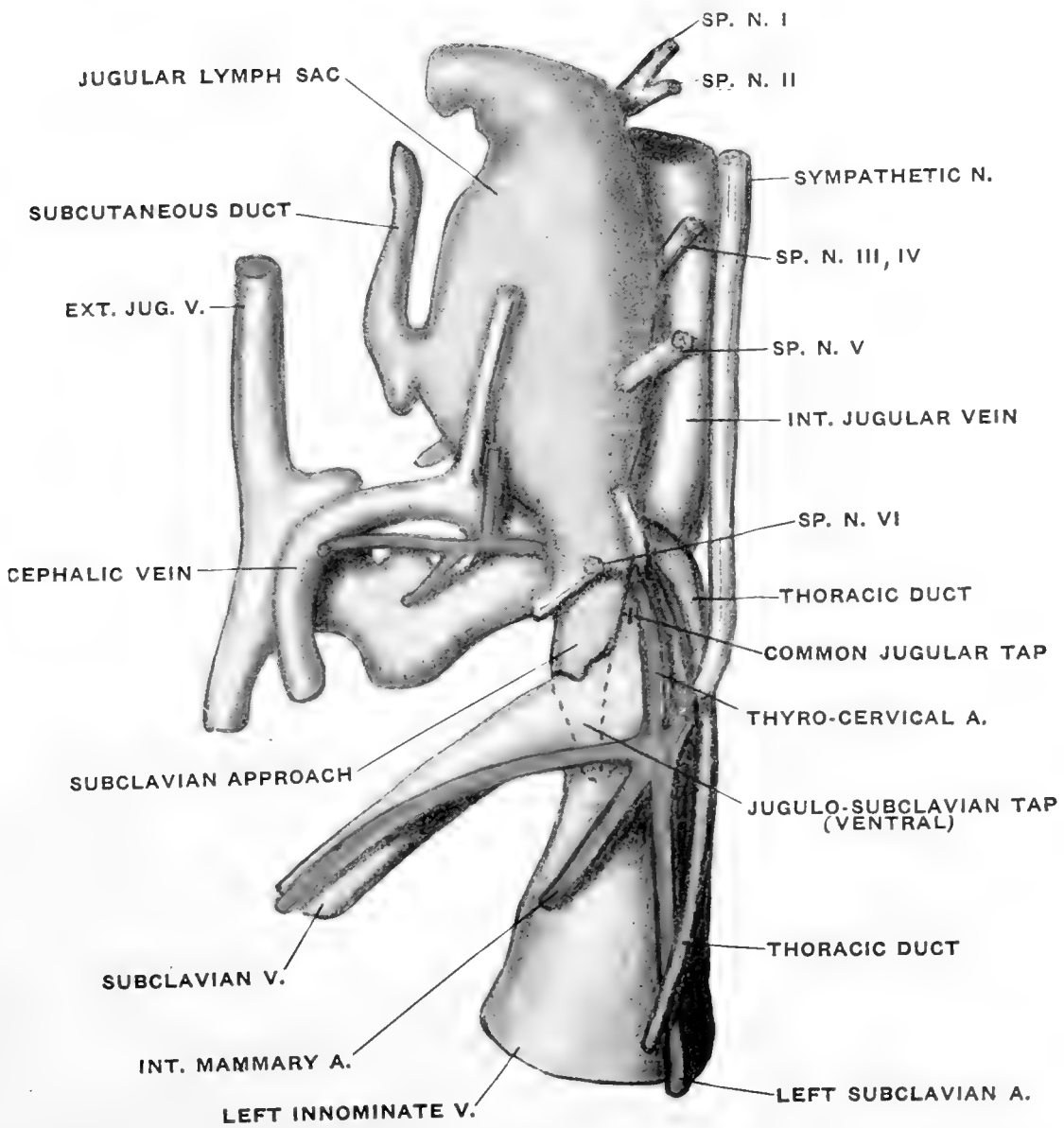
59


JUGULAR LYMPH SAC
SP. N. V
SUBCUTANEOUS DUCT
CAROTID A.
SYMPATHETIC N.
INTERNAL JUGULAR (PRECARDINAL)
CEPHALIC VEIN
PROXIMAL END THORACIC DUCT
4S
SUBCLAVIAN APPROACH
SP. N. VI
THYRO-CERVICAL A.
INNOMINATE A.
PRIMITIVE ULNAR LYMPHATIC
SUBCLAVIAN A.
AORTIC ARCH
INT. MAMMARY A.

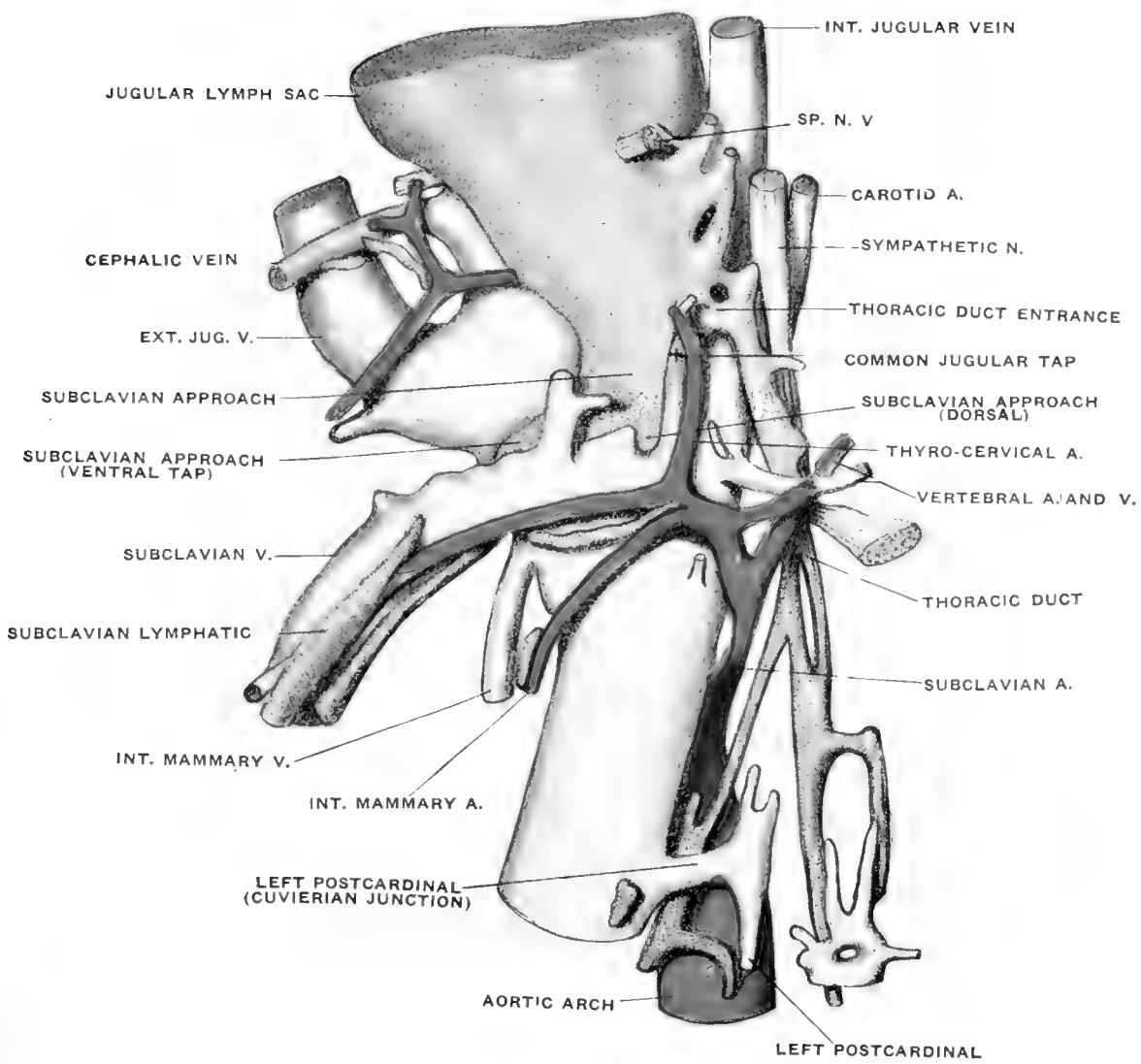
60

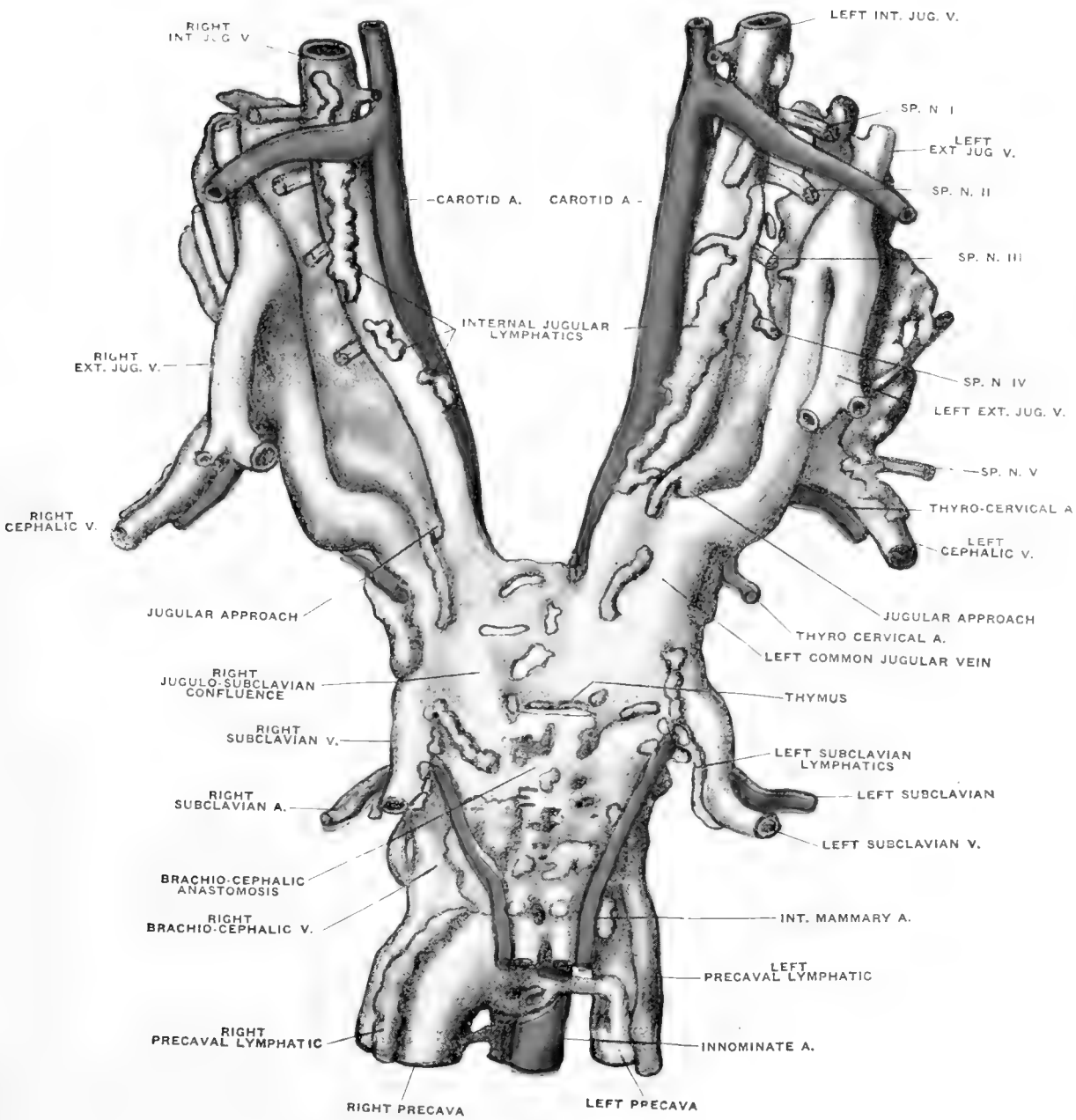

EXT. JUG. V.
JUGULAR LYMPH SAC
INT. JUGULAR VEIN
VAGUS N.
SUBCUTANEOUS DUCT
CAROTID A.
SP. N. V
JUGULAR APPROACH
CEPHALIC VEIN
COMMON JUGULAR TAP
SP. N. VI
SUBCLAVIAN APPROACH
INNOMINATE A.
PRIMITIVE ULNAR LYMPHATIC
INT. MAMMARY A.
AORTIC ARCH
INT. MAMMARY V.
SUBCLAVIAN V.

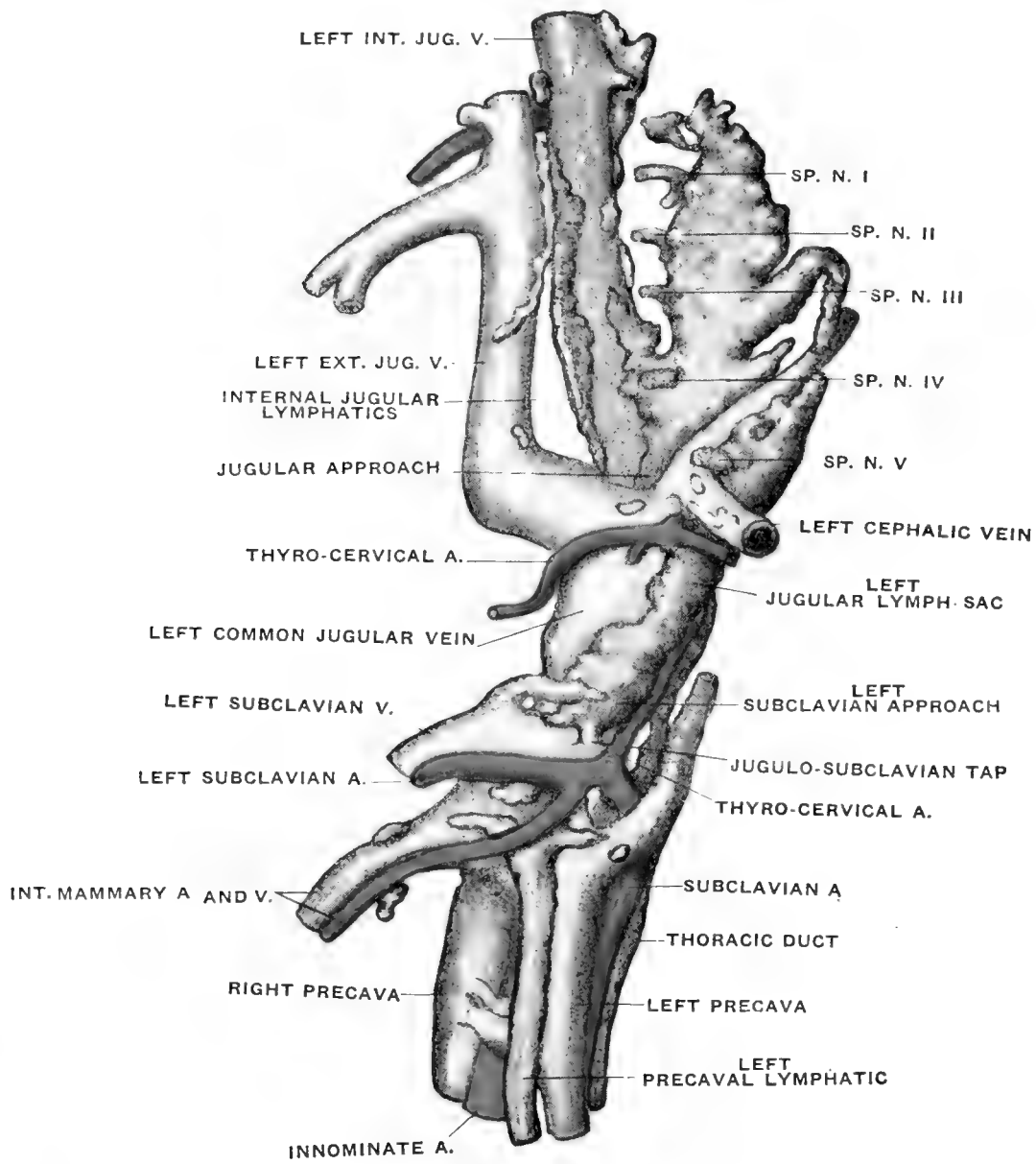
61

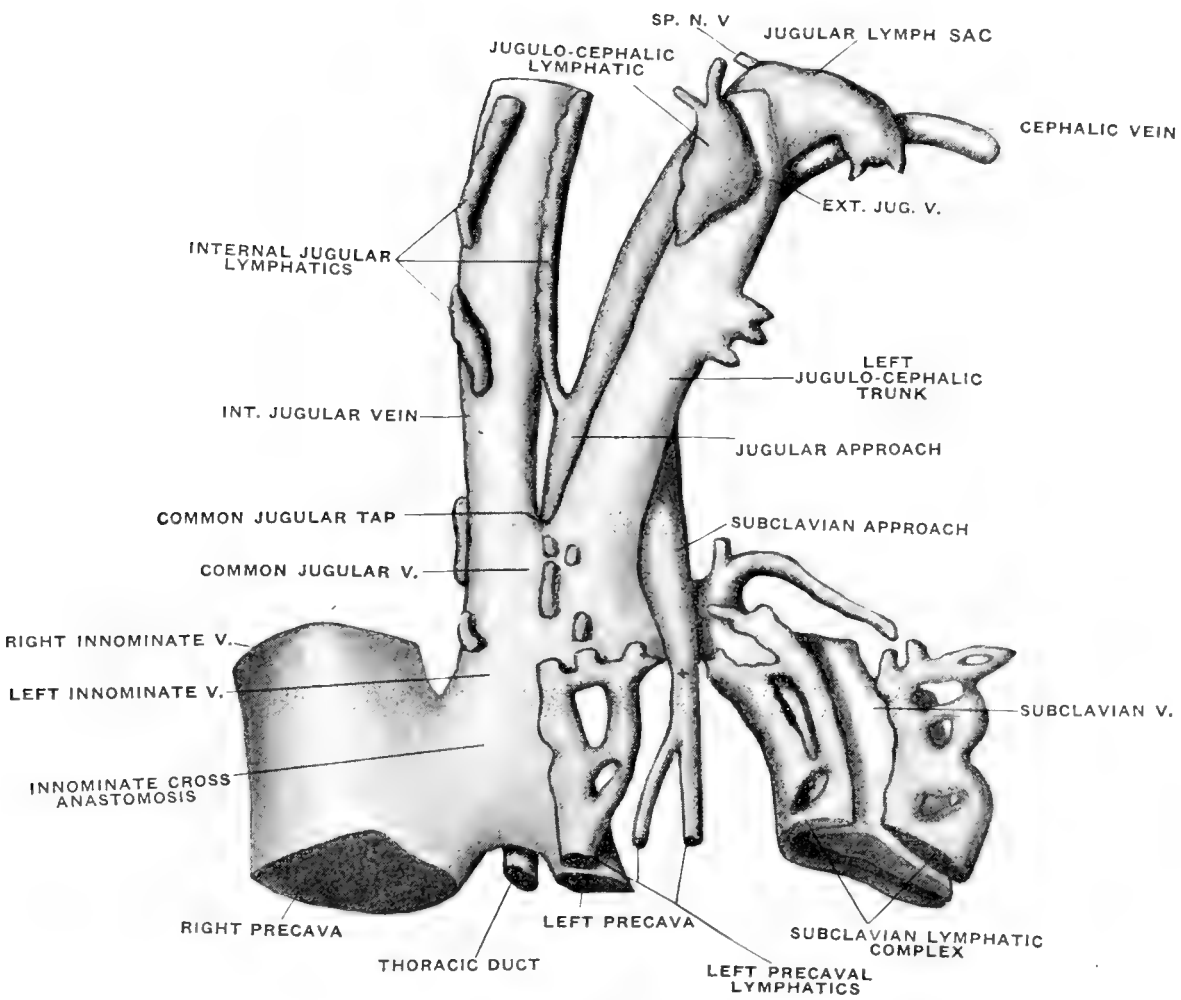

SP. N. I
JUGULAR LYMPH SAC
SP. N. II
SYMPATHETIC N.
SUBCUTANEOUS DUCT
SP. N. III, IV
EXT. JUG. V.
SP. N. V
INT. JUGULAR VEIN
SP. N. VI
CEPHALIC VEIN
THORACIC DUCT
COMMON JUGULAR TAP
THYRO-CERVICAL A.
SUBCLAVIAN APPROACH
JUGULO-SUBCLAVIAN TAP (VENTRAL)
THORACIC DUCT
SUBCLAVIAN V.
INT. MAMMARY A.
LEFT SUBCLAVIAN A.
LEFT INNOMINATE V.

62


INT. JUGULAR VEIN
JUGULAR LYMPH SAC
SP. N. V
CAROTID A.
SYMPATHETIC N.
CEPHALIC VEIN
THORACIC DUCT ENTRANCE
EXT. JUG. V.
COMMON JUGULAR TAP
SUBCLAVIAN APPROACH
SUBCLAVIAN APPROACH (DORSAL)
SUBCLAVIAN APPROACH (VENTRAL TAP)
THYRO-CERVICAL A.
VERTEBRAL A. AND V.
SUBCLAVIAN V.
THORACIC DUCT
SUBCLAVIAN LYMPHATIC
SUBCLAVIAN A.
INT. MAMMARY V.
INT. MAMMARY A.
LEFT POSTCARDINAL (CUVIERIAN JUNCTION)
AORTIC ARCH
LEFT POSTCARDINAL


RIGHT INT. JUG. V.
LEFT INT. JUG. V.
SP. N. I
LEFT EXT. JUG. V.
SP. N. II
SP. N. III
CAROTID A.
CAROTID A.
INTERNAL JUGULAR LYMPHATICS
RIGHT EXT. JUG. V.
SP. N. IV
LEFT EXT. JUG. V.
SP. N. V
THYRO-CERVICAL A.
RIGHT CEPHALIC V.
LEFT CEPHALIC V.
JUGULAR APPROACH
JUGULAR APPROACH
THYRO CERVICAL A.
LEFT COMMON JUGULAR VEIN
RIGHT JUGULO-SUBCLAVIAN CONFLUENCE
THYMUS
RIGHT SUBCLAVIAN V.
LEFT SUBCLAVIAN LYMPHATICS
RIGHT SUBCLAVIAN A.
LEFT SUBCLAVIAN
LEFT SUBCLAVIAN V.
BRACHIO-CEPHALIC ANASTOMOSIS
RIGHT BRACHIO-CEPHALIC V.
INT. MAMMARY A.
LEFT PRECAVAL LYMPHATIC
RIGHT PRECAVAL LYMPHATIC
INNOMINATE A.
RIGHT PRECAVA
LEFT PRECAVA


LEFT INT. JUG. V.
SP. N. I
SP. N. II
SP. N. III
LEFT EXT. JUG. V.
SP. N. IV
INTERNAL JUGULAR LYMPHATICS
JUGULAR APPROACH
SP. N. V
LEFT CEPHALIC VEIN
THYRO-CERVICAL A.
LEFT JUGULAR LYMPH SAC
LEFT COMMON JUGULAR VEIN
LEFT SUBCLAVIAN V.
LEFT SUBCLAVIAN APPROACH
JUGULO-SUBCLAVIAN TAP
LEFT SUBCLAVIAN A.
THYRO-CERVICAL A.
INT. MAMMARY A AND V.
SUBCLAVIAN A
THORACIC DUCT
RIGHT PRECAVA
LEFT PRECAVA
LEFT PRECAVAL LYMPHATIC
INNOMINATE A.


SP. N. V
JUGULAR LYMPH SAC
JUGULO-CEPHALIC LYMPHATIC
CEPHALIC VEIN
EXT. JUG. V.
INTERNAL JUGULAR LYMPHATICS
LEFT JUGULO-CEPHALIC TRUNK
INT. JUGULAR VEIN
JUGULAR APPROACH
COMMON JUGULAR TAP
SUBCLAVIAN APPROACH
COMMON JUGULAR V.
RIGHT INNOMINATE V.
LEFT INNOMINATE V.
SUBCLAVIAN V.
INNOMINATE CROSS ANASTOMOSIS
RIGHT PRECAVA
LEFT PRECAVA
SUBCLAVIAN LYMPHATIC COMPLEX
THORACIC DUCT
LEFT PRECAVAL LYMPHATICS

THE SINUS MAXILLARIS AND ITS RELATIONS IN THE EMBRYO, CHILD, AND ADULT MAN.

BY

JACOB PARSONS SCHAEFFER,

Instructor in Anatomy, Cornell University Medical College, Ithaca, N. Y.

WITH 31 FIGURES.

This paper is based upon a study of the sinus maxillaris in the embryo in successive stages up to the fetus at term, as well as in the child and adult man. Most of the adult specimens ranged in age from 18 to 80 years.

The lateral nasal wall of the embryo, at different stages of growth, was modeled; thus showing the relations of the sinus maxillaris and its progress in development. The blotting-paper method was used in all the reconstructions. Both solid structures and cavities were modeled, thus securing positive and negative views.

The work also covers a general consideration of the sinus in the child and the adult; including a study of the sinus relations, its ostium or aperture, and the ostium accessorium. Special attention was also given to the cause and effect of recesses occurring on the walls of the adult sinus. Dissections were made to cover all phases of the problem.

In determining the size of the sinus the following measurements were taken: (1) dorsosuperior diagonal; (2) ventrosuperior diagonal; (3) superoinferior; (4) ventrodorsal; (5) mediolateral.

The capacity of the sinus was determined by filling the cavity with a portion of a previously measured liquid, or by measuring the amount of water the hardened mucous membrane (representing the exact shape and size of the sinus) would displace.

I wish to take this opportunity for expressing grateful acknowledgment to the heads of the Departments of Anatomy, and Embryology and Histology for valuable criticisms and helpful

suggestions. I also wish to express my grateful appreciation of the abundant material and other facilities placed at my disposal by the aforementioned departments. To Professor and Mrs. Gage, for the loan of embryos from the research collection, I wish to express thanks.

THE EMBRYOLOGY AND EARLY RELATIONS OF THE SINUS MAXILLARIS.

About the tenth week of fetal life the mucous membrane in the primitive middle meatus of the nose begins to pouch laterally. This pouch represents the Anlage of the sinus maxillaris, which pushes from the originally simple furrow separating the maxillo-turbinal (later concha nasalis inferior) and the first ethmo-turbinal (later concha nasalis media).

In order to gain a clearer conception of the location and relations of this primary maxillary pouch, and to better interpret adult conditions, a brief consideration of the lateral nasal wall, of the embryo, is necessary.

During the second month of intrauterine life, before the cavum nasi and the cavum oris have become separate cavities, we find three swellings on the lateral wall of the nasal fossa (maxillo-turbinal, appearing first; ethmo-turbinal, appearing next; naso-turbinal –extremely rudimentary in man, appearing later) (fig. 1). The maxillo-turbinal corresponds to the adult concha nasalis inferior. The naso-turbinal, which is termed by Peter, "der Agger nasi," and by Killian, in conjunction with the primitive processus uncinatus, "der erste Hauptmuschel," persists in the adult as the agger nasi. The ethmo-turbinal undergoes subdivision, and by this division, according to Killian, five ethmo-turbinal plates, defined by six grooves, are usually formed. E. Kallius says, "Dass alle diese 6 Furchen ausgebildet sind, ist selten." Zuckerkandl's investigations show that three ethmo-turbinal plates are the typical number. He says, "Drei Siebbeinmuscheln repräsentieren demnach die typische Faltungsweise des Siebbeines." According to the embryos studied for this paper, I find that the number of furrows and ethmoidal conchæ varies, but in the specimens examined, four plates are rather

common. The ethmo-turbinal plates and the resultant furrows become reduced in number as development goes on, and finally represent the conchæ nasales, media and superior, and the meatus nasi, medius and superior, respectively. The reduction in number may not be carried so far, and this accounts for the supernumerary ethmoidal conchæ and meatus in many adults.

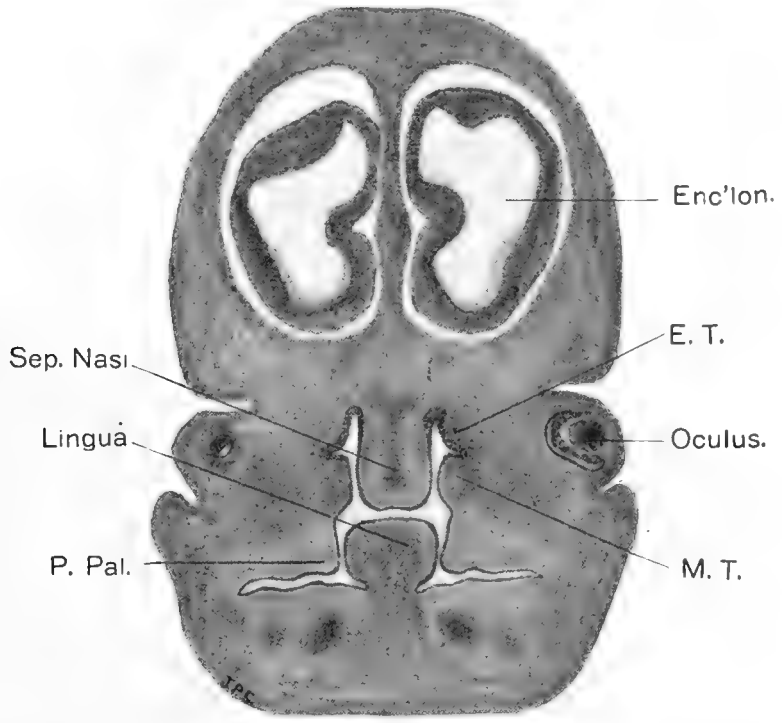


Fig. 1 (× 10). Drawing of a frontal section through the head of an embryo aged about 45 days, in the region immediately dorsal to the organon vomeronasale (Jacobsoni), note the development of the early turbinal processes, and that there is no cartilage laid down in them at this early period. This is contradictory to the theory that the turbinal processes are primarily the result of an inpushing of the lateral nasal wall by cartilaginous strands which later become the nasal conchæ.

Enc'lon, = encephalon; **E. T.,** = ethmo-turbinalia; **M. T.,** maxillo-turbinale; **P. Pal.,** = processus palatinus; **Sep. Nasi,** = septum nasi.

Just how the primitive nasal processes and furrows are formed is interpreted differently. Some claim that the projections are due to an inpushing of the lateral nasal wall by the cartilaginous strands which become the nasal conchæ. This latter claim I have been unable to verify, because I find that the folds or projections are *invariably present before cartilage is found in them*

(fig. 1). The elevations at first consist of a duplication of the ectoderm filled with indifferent mesenchyma, which, in part, later changes into cartilage. The nasal conchal cartilages are, therefore, a result and not a cause of the early condition. Schoenemann claims that they are elevations left by excavations of furrows on the lateral wall of the nasal fossa. Killian and Mihalkovics hold that the projections are free ingrowing folds on the lateral wall of the nasal fossa. Glas concludes a discussion on the nasal conchæ in rats thus:

Die Bildungsmodus der Muscheln, ist die Resultierende zweier Komponenten; (1) des Auswachsens die Wandpartien einwachsender Epithelleisten (Fissuren). (2) des Vorwachsens bestimmter Wandpartien.

After a study of these early conditions I am led to believe that the *primitive* furrows are *primarily* the result of an outpushing or outgrowing of the mucous membrane on the lateral wall of the nasal fossa. The projections, by duplication of the mucous membrane (especially true in the ethmo-turbinal region) (fig. 1), and the deepening of the furrows, become rapidly prominent. At times the two processes, an outgrowth or outpushing, and a duplication of the intervening mucous membrane and mesenchyme, seem to be at work simultaneously in forming the early projections and furrows. The theory that the furrows are *primarily* started as an outpushing or outgrowing of mucous membrane is entirely in accord with, apparently, similar processes taking place in the early embryo nose; namely, the pouching or outgrowing of the mucous membrane as the *Anlagen* of some of the sinus paranasales. That a similar process should cause the formation of *primarily* similar outgrowths seems plausible.

It is, however, not the province of this paper to speak in detail of the development of the early projections and furrows; suffice it to say that it is from the furrow separating the primitive conchæ nasales, inferior and media, that the maxillary pouch evaginates. It is, therefore, the primitive meatus nasi medius with its contained structures, and the naso- and first ethmo-

turbinals that especially concern us in the development and relations of the primitive sinus maxillaris.

Killian terms the naso-turbinal and the subdivisions of the ethmo-turbinal, "Hauptmuscheln;" and the smaller projections appearing in the furrows between these "Hauptmuscheln," as "Nebenmuscheln." What he terms "die zweite Hauptmuschel" will be spoken of in this paper as the concha nasalis media (first ethmoidal concha).

The naso-turbinal plus the processus uncinatus and the concha nasalis media have marked bends, thus presenting ascending and descending crura. Correspondingly the furrow between these conchæ has a bend, and presents ascending and descending limbs. For the sake of description we will consider the processus uncinatus as the descending crus of the naso-turbinal. The primitive meatus nasi medius has, therefore, as inferior boundaries the cura of the naso-turbinal and the space existing between the conchæ nasales, media and inferior. The superior boundaries of the space are the crura of the concha nasalis media (zweite Hauptmuschel of Killian) (fig. 4). To say then, as has been done earlier in this paper, that the maxillary pouch evaginates from the space separating the primitive conchæ nasales, inferior and media, is not giving the pouch its *definite* location.[1] The actual point of this primary pouching is from the primitive infundibulum ethmoidale, or the "unterer Recessus des absteigenden Astes der ersten Hauptfurche" of Killian.

This pouch is a minute epithelial sac, and forms the Anlage of the sinus maxillaris. Its *earliest establishment* precedes the appearance of the cartilage which later surrounds it. This is in accord with the statement of E. Kallius, that

> . . . . . die Nebenhöhlen der Nase schon angelegt sind, ehe der Knorpel entsteht, und dass also das Skelett sich erst sekundär um jene herumlegt.

[1]The relation of the space existing between the conchæ nasales, inferior and media, to the descending ramus or limb of the first furrow is spoken of thus by Killian, "Der Raum zwischen zweiter Hauptmuschel und unterer Muschel ist demnach nur eine Art Vorhof zum absteigenden Theil der ersten Haupfurche."

According to my observations the *earliest* evidences of maxillary pouching are found about the seventieth day of fetal life. Kallius places the time of the primary evagination during the middle of the third month, "Die Oberkieferhöhle erscheint in der Mitte des 3. Monats." J. Kollman places the time of pouching later, "Seine Anlage beginnt erst bei Foeten Von 8 cm. Länge." Gegenbaur quotes Dursy as authority for the following:

> Schon bei 8 cm. lange Embryonen buchtet sich der Raum der Nasenhöhle zwischen mittlerer und unterer Muschel gegen den hier verdickten Knorpel der Seitenwand der Nasenhöhle aus und bildet die Anlage des Sinus Maxillaris.

I have found the primitive maxillary pouch duplicated, i. e., two pouches growing laterally side by side. (This may explain some of the duplications of the adult ostium maxillare—the two primary pouches fusing distally, leaving the two points of evagination as the ostia maxillaria of the adult sinus. Other duplications of the adult ostium may be caused in a manner similar to the formation of the accessory ostium).

This embryonal condition probably explains some of the cases in which the sinus maxillaris is divided into two partially or wholly separate compartments by a vertical partition, i. e., each pouch developing into an adult cavity independent of its mate (see subsequent paragraph).

The primary ostium maxillare varies greatly in its dimensions in different embryos (figs. 2, 3). This is entirely in accord with adult conditions, since the ostium of the adult sinus has a great range of dimensions (table D). The great differences in the dimensions of the ostium may be due to early fusion of two or more primary maxillary pouches; or the primitive pouching may have been single but extensive, as is frequently the case. These two latter would give rise to long slit-like ostia, while the single and less extensive pouching would give us the typically shaped and average sized adult ostium.

Sometime prior to the establishment of the maxillary pouch[2]

[2] It is indeed difficult, in some cases, to say which structure is the primary one in establishing an *Anlage*. It most cases the processus uncinatus is the first to

a ridge appears immediately inferior to the point of maxillary evagination. This ridge is the Anlage of the processus uncinatus and, as said before, will be considered, merely for the sake of description, as the descending crus of the naso-turbinal. It will be recalled that Killian terms the latter two structures, "die

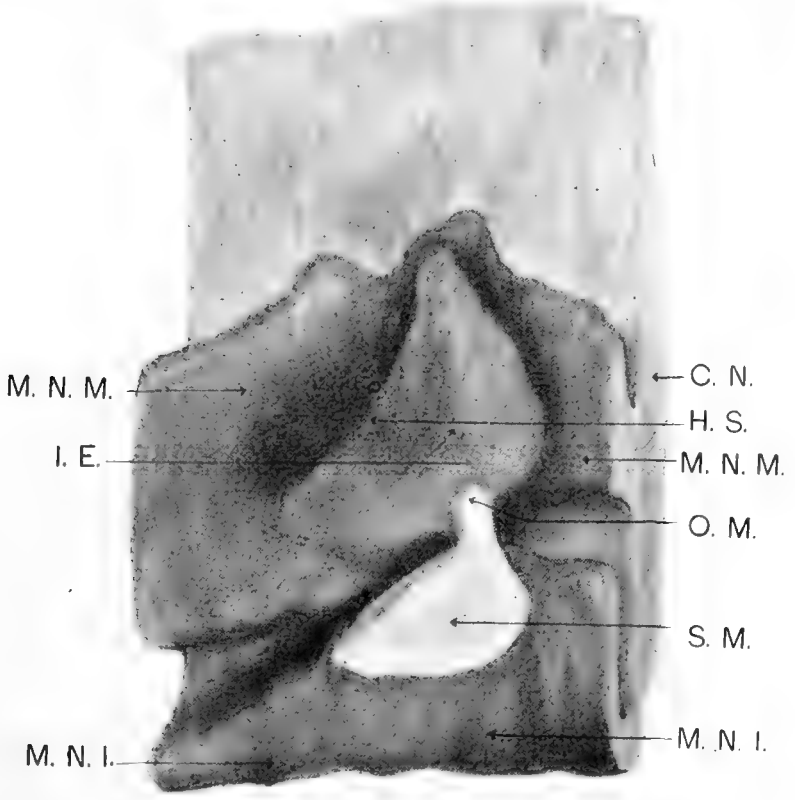


Fig. 2 (×10). Drawing of a reconstruction of portion of the right nasal cavity; including the meatus, infundibulum ethmoidale, and the sinus maxillaris. Only that portion of the nasal cavity necessary to include the sinus maxillaris and its relations is shown.

Compare the size of the ostium maxillare with the corresponding aperture in fig. 3.

The model was reconstructed from the nose of an embryo aged 105 days. It must be remembered that the drawing represents cavity and is, therefore, a negative.

M. N. M., M. N. I., =meatus nasi, medius et inferior; **I. E.,** = infundibulum ethmoidale; **C. N.,** = cavum nasi; **H. S.,** = hiatus semilunaris; **O. M.,** = ostium maxillare; **S. M.,** =sinus maxillaris.

appear, and in some instances it is impossible to say whether the pouching of the mucous membrane, or the formation of the ridge is first. It may, however, be said that both structures are more or less dependent upon each other in establishing *Anlagen*.

erste Hauptmuschel." This ridge has its free border directed superiorly, and it extends in a ventrosuperior direction. It early tends to form a shallow groove immediately superior to it, which is the primitive infundibulum ethmoidale (Recessus inferior des absteigenden Astes der ersten Hauptfurche of Killian). To be

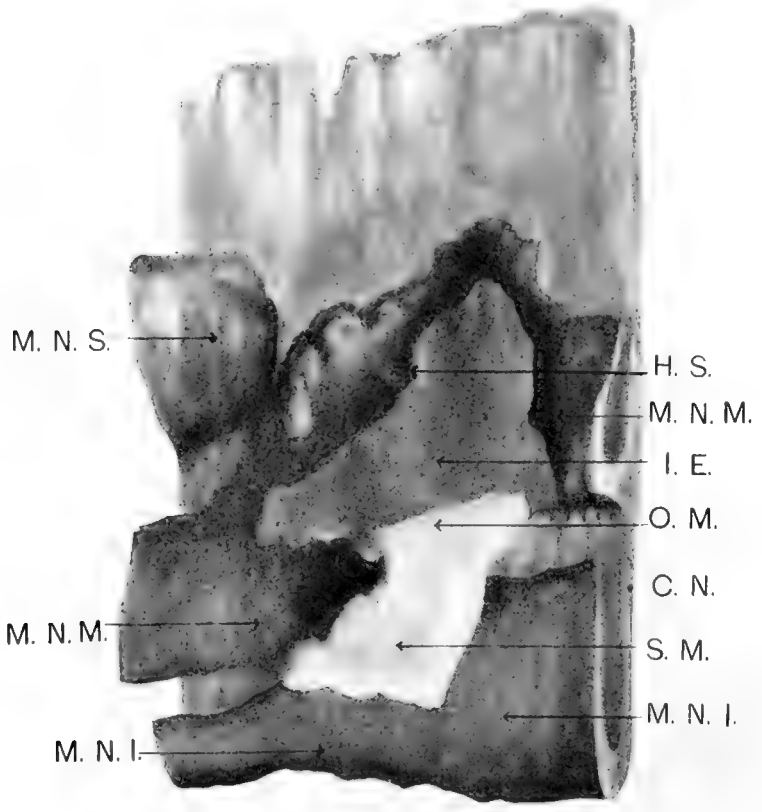


Fig. 3 (× 10). Drawing of a reconstruction of portion of the right nasal cavity of an embryo aged 120 days. The drawing includes the meatus, infundibulum ethmoidale, and the sinus maxillaris. Only that portion of the cavity, was modeled so as to include the sinus maxillaris and its relations. Since it represents cavity it is a negative.

Note the very extensive ostium maxillare in comparison to that in fig. 2.

M. N S., = meatus nasi superior; **M. N. M., M. N. I.**, = meatus nasi, medius he inferior; **I. E.**, = infundibulum ethmoidale; **C.N.**, = cavum nasi; **H. S.**, = ttaius semilunaris; **O. M.**, = ostium maxillare; **S. M.**, = sinus maxillaris.

accurate, then, we must say that the maxillary pouch evaginates from the primitive infundibulum ethmoidale —a part of the meatus nasi medius.

Some time after the appearance of these structures there is a more or less uneven bulging on the lateral wall of the primitive

meatus nasi medius, immediately superior and lateral to the free border of the processus uncinatus (fig. 4). This is the Anlage of the bulla ethmoidalis. The slit-like space existing between the free border of the processus uncinatus and the bulla ethmoidalis is the primary hiatus semilunaris. Through this slit the infundibulum ethmoidale communicates directly, and the primitive sinus maxillaris indirectly, with the meatus nasi medius.

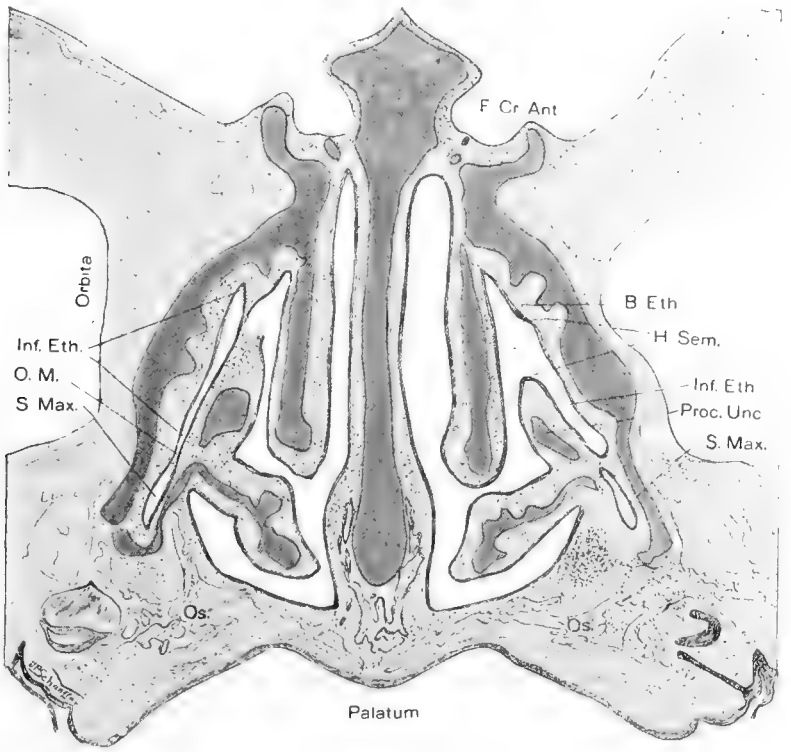


Fig. 4 (×6.6). Drawing of a frontal section of the nose of an embryo aged 120 days. The section is 7.25 mm. from the tip of the nose. Note that on one side the section is in the region of the ostium maxillare, on the other it is dorsal to it; also the fusion between the processus uncinatus and a frontal concha.

Inf. Eth., = infundibulum ethmoidale; **O. M.**, = ostium maxillare; **S. Max.**, = sinus maxillaris; **Os.**, = developing bone; **F. Cr. Ant.**, = fossa cranii anterior; **B. Eth.**,=bulla ethmoidalis; **H. Sem.**, = hiatus semilunaris; **Proc. Unc.**, = processus uncinatus.

Killian subdivides the uneven thickening on the lateral wall of his "Ramus decendens der ersten Hauptfurche" into small projections with very shallow intervening furrows. The projections are his "absteigende Nebenmuscheln", and the furrows the "obere und untere Zwischenfurchen" of the first furrow, or the *primitive* meatus nasi medius. He concludes that the bulla is formed by

the early fusion of some of the processes (Nebenmuscheln). The space immediately inferior to his "untere Nebenmuschel" or the space he designates as the "Recessus inferior" of his first chief furrow, is the infundibulum ethmoidale—the exact place of the primary maxillary pouching.

At this juncture mention must be made of the primary pouching of the sinus frontalis in order to interpret later conditions in connection with adult fronto-maxillary relations. It will be remembered that the furrow from which the maxillary pouch evaginates has ascending and descending rami, and that from the descending ramus the maxillary evagination takes place. The ascending ramus of this furrow widens and pushes ventrally and superiorly. Turner says:

> It is generally held that the frontal sinus commences to develop at the end of the first or the beginning of the second year of life, as an upward expansion of the ethmoid cell labyrinth.

Hartman quotes Steiner for the following:

> Der erste Anlage der Stirnhöhle ist in der Anlage des Knorpeligen Siebbein-labyrinthes gegeben mit der Entwickelung der zelligen Räume des vorderen Siebbeinlabyrinthes beginnt auch die der Stirnhöhle, denn letztere stellt eben nur die Ausdehnung der vorderen Siebbeinzellen nach oben dar.

Hartman makes the following statement:

> Aus dem aufsteigenden Ast der ersten Hauptfurche bildet sich durch oberflachliche Verwachsung eine sackartige Bucht, der Recessus ascendens od. R. frontalis, die Stirnbucht. Aus der Stirnbucht entwickelt sich die Stirnhöhle.

The embryos studied showed evidence of a slight pouching at the superior and ventral end of the primitive meatus nasi medius. This doubtless corresponds to the "Recessus frontalis" of the first chief furrow of Hartman and Killian.

According to Killian's commendable work there are three processes and four furrows on the lateral wall of the recessus frontalis, which are designated by him as "Stirnmuscheln und Stirnfurchen," respectively. The processes, according to Killian,

merge, and form the cellulæ ethmoidales anterior, or cellulæ frontales as some call them. From this he concludes that the sinus frontalis may continue its development in one of the following ways: (1) by extension of the frontal recess (direct method), (2) by extension of a frontal cell (indirect method), (3) by extension of the frontal recess and a frontal cell, (4) by extension of two frontal cells. With these facts kept in mind—allowing for further differentiation during development, it is easier to understand why the sinus frontalis, in the adult, connects either with the infundibulum ethmoidale, with the meatus nasi medius, or with both. These embryological facts are of importance in connection with adult fronto-maxillary relations. Doubtless more work should be done on the development of the nasofrontal duct in order to clear up some points in connection with the relations existing between the sinus frontalis and maxillaris. I am now working along this line and hope to report my findings at some future time.

Although the pouching to form the recessus frontalis, or what may be termed the Anlage of the sinus frontalis, begins during the third month of fetal life, as does that of the sinus maxillaris, the extension of the sinus frontalis is for a time so small that it is usually regarded as wanting at birth. This is in part due to the fact that the sinus frontalis is as a rule looked for in the frontal or vertical portion of the frontal bone, while the first evidences of it are to be sought elsewhere. In fact, according to Lothrop's investigations, the sinus frontalis of the adult does not reach the vertical or frontal portion of the frontal bone in about three per cent of cases—the only evidences of the sinus appearing in the horizontal or orbital portion of the frontal bone. It must, however, be said that the sinus frontalis is *tardy* in its development until after birth; while, on the other hand, the pouch forming the *Anlage* of the sinus maxillaris develops more rapidly and occupies a definite space in the lateral wall of the nasal fossa by the end of the third fetal month (figs. 2, 3).

By the simultaneous processes of resorption of surrounding tissue and the growth of the maxillary pouch, the primitive cavity gains more amd more capacity. The pouch soon acquires

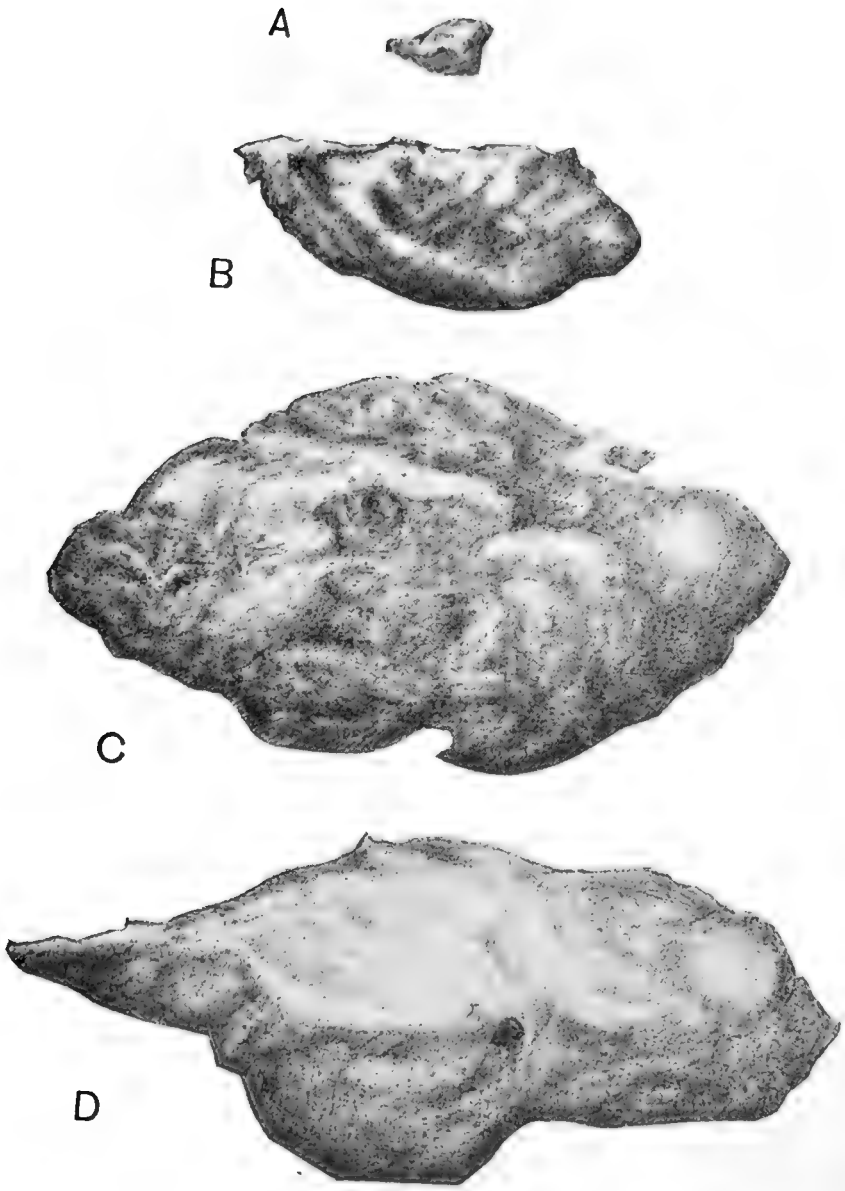


FIG. 5 (× 4). Drawings of the mucous membrane, representing the exact shapes of the sinus maxillaris at different stages of its development in the fetus and child.

A. From a fetus aged 4 months. **B.** From a fetus at term. **C.** From a child aged 18 to 20 months. **D.** From a child aged 20 to 23 months.

a slit-like shape at the side of the nose (fig. 4). It has its greatest measurement in the ventrodorsal direction, while mediolaterally the cavity occupies comparatively little space. In embryos aged from 100 to 105 days the ventrodorsal measurement is about 2 mm. (fig. 2). In a 120-day embryo the distance is about 2.5 mm. (figs. 3 and 5 A). In a 100-day embryo the most ventral spur of the sinus is about 6.5 mm., and the most dorsal spur 8.5 mm. from the tip of the nose.

It will be remembered that, in the embryo, the processus alveolaris of the maxilla is in proximity to the orbit, and when we recall the fact that the unerupted teeth are contained in this situation, it at once becomes evident that the sinus maxillaris must be correspondingly small at this time. Because of these facts the sinus of a 7-month fetus measures only 5 mm. in the ventrodorsal plane, while that of a fetus at term has increased this distance to approximately 7 mm. (fig. 5 B). During the latter month of intrauterine life the sinus gains in the mediolateral plane, so that at term this distance measures from 3 to 4 mm.

It is generally stated that the deciduous teeth hold the sinus maxillaris in check, and that the cavity rapidly assumes larger dimensions as the first dentition progresses. I, however, find that the growth of the sinus is rather uniform, and that the first dentition has little to do with any rapid increase in the size of the cavity. *The age of the child and the size of the sinus, apparently, progress pari passu* (fig. 6).

The ventrodorsal measurement of the sinus in a child aged 6 months is 10 mm., but the cavity has not developed sufficiently in the mediolateral plane to reach beneath the orbit. In a child of 9 months the ventrodorsal distance is 14 mm., with a superoinferior measurement of 5 mm. At the end of the first year the sinus has reached a ventrodorsal measurement of 16 mm., a superoinferior of 6 mm.; and has now reached a mediolateral point sufficient to pass beneath the orbit. As the maxilla grows, the sinus remains for some time on the medial side of the infraorbital canal (fig. 10). By the twentieth month the sinus measures ventrodorsally 20 mm. (fig. 5) and has, as a rule, extended above the rudimentary first permanent molar tooth.

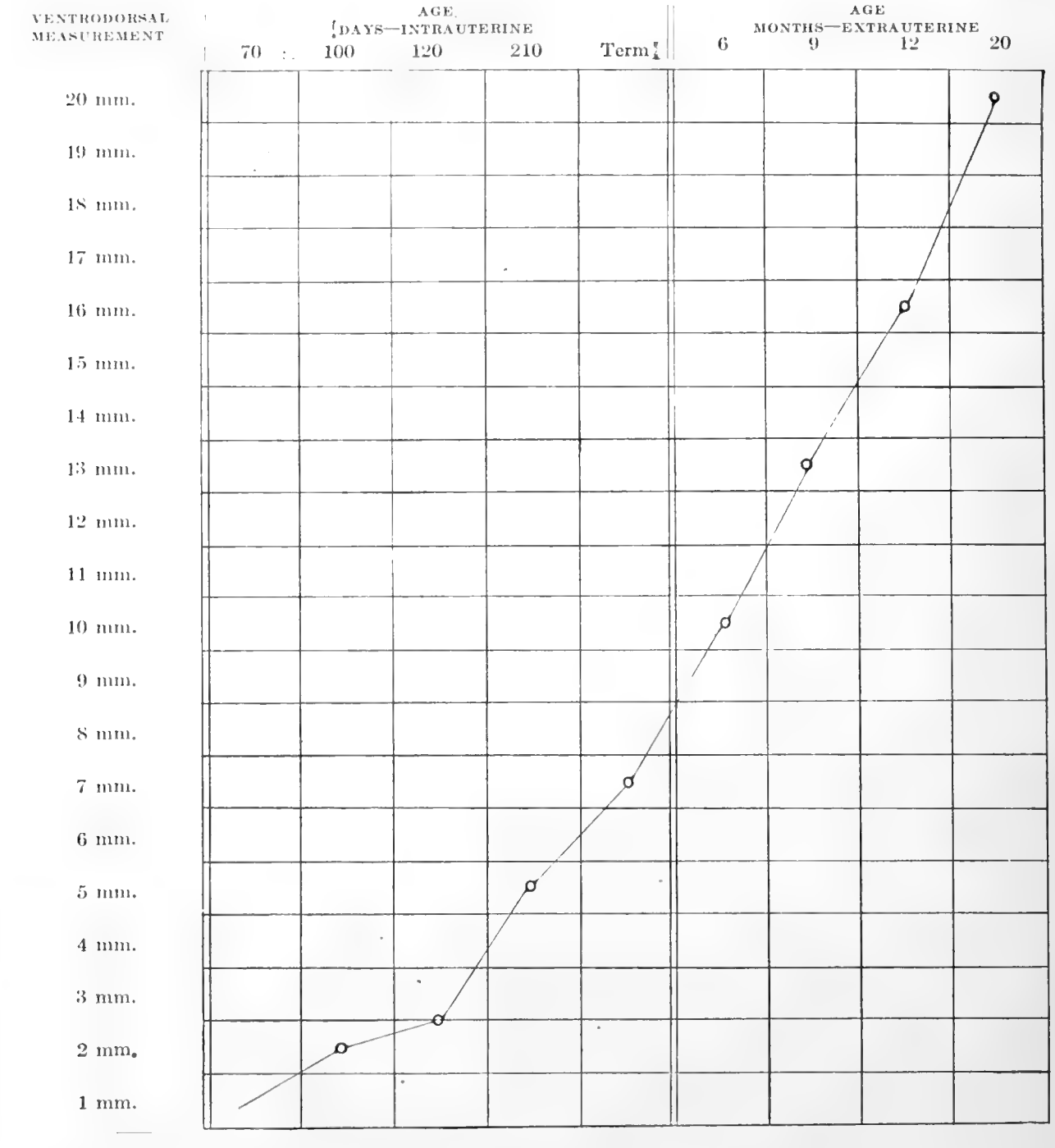


Fig. 6. Chart showing the gradual increase in size of the sinus maxillaris in its ventrodorsal plane.

Before and during dentition the sinus maxillaris is separated from the front of the maxilla by the unerupted teeth (fig. 17). After the eruption of the deciduous teeth the cavity continues to have a more or less rounded and elongated shape (fig. 5 D). It is really never spherical as is often stated, but has an irregular elongated form from the beginning.

After the eruption of the permanent teeth the sinus begins to lose its rounded and elongated shape and to assume the adult pyramidal form (figs. 7, 8, and 9). By the twelfth or fifteenth year of age, when the second molar has appeared, the sinus approaches, though it has not yet attained its definite shape. The sinus reaches its full size between the fourteenth and eighteenth year.

THE ADULT SINUS MAXILLARIS.

The adult sinus maxillaris was known to Galenus (130-201), but apparently Dr. Nathaniel Highmore was the first to give any detailed description of it. In his work (1651), "Corporis Humani Disquisitio Anatomica," he describes the cavity in the maxilla, to which his attention was drawn by a lady patient, in whom an abscess of this cavity, since known as the antrum of Highmore (sinus maxillaris), was drained by the extraction of the canine tooth (left). The following are the exact words of Doctor Highmore in describing the cavity located in the body of the maxilla. His report of the case also follows.

"ANTRUM MAXILLÆ SUPERIORIS."

Antrum hoc utrinque unum, sub oculi sede inferiore ubi os ad oculi tutelam quodammodo protuberat, ad latera inferiora nasi situm est. Insigniter cavum sphaericum, aliquantulum vero oblongum, et ita amplum ut articulus pollicis majoris pedis ultimus in illo delitescat, ... Osse attenuata seu squammâ osseâ obtegitur: Os enim quod illud includit, et quod a dentium alveolis extremis distinguit, crassitie chartam Emporeticam non multum excedit. . . . . In basi hujus protuberantes quaedam eminentiae cernuntur, ... Quibus dentium apices tenniores includuntur. . . . . Dentium alveoli margini hujus ossis inferiori insculpuntur, quibus dentes in-

figuntur. . . . . Antrum hoc frequentius vacuum, aliquando muco repletum reperitur, in quod humores a capite per meatum quendam a cavitate illa in osse frontis, et ab osse ethmoeide destillare poterunt. . . . . .

Atque hic silentio praeterire non possumus, quod generosae cuidam foeminae sub nostra cura laboranti accedit. Cum sub ferina eaque continua falsi humoris distillatione, per multos retro annos laborasset, omnesque pené dentes corrosos ac cariosos evulserat; nec tamen a dolore liberata, tandem dente canino sinistri lateris effosso . . . . Simul squammosa illa distinctio inter cavitatem hanc et dentis foveam criptur, adeo ut humorum, per alveolum dicti dentis, ab antro illo perrenis successerit destillatio; Qua multum perterrita, stylo argenteo in alveolum immissa originem fontis hujus exploratura, usque ad oculum, per uncias pené duas sursum adegit; magis adhuc metuens, pennam minorem plumis decerptis totam pené ad longitudinem palmae unius immisit. Iam maximae consternata, ad Cerebrum usque decurrere existimans, me inter alias consulit; ubi autem singulas examinavimus circumstantias, pennae reduplicationes, illamque per cavitatem hanc circumgyrare invenimus. Atque sic, ubi in figura seguenti cavitatem hanc designavimus, illam de usu ac necessitate hujus satis instructam, perennisque illius fontis patentissimam habuimus, a timore et medicina simul desistit.

Antrum hoc levitatis ossium causa, quae hic oculorum situs gratia crassa esse debuere, factum esse arbitramur.

The antrum (sinus maxillaris) described by Highmore must have been an exceptionally large one, because the canine tooth does not as a rule come in relation with the sinus. This same tooth is mentioned by some writers even today as the tooth to extract in draining the sinus. It is a very bad tooth to select for this purpose in the great majority of cases. This fact will be referred to in a subsequent paragraph when considering teeth relations. It will also be noticed that Highmore had a somewhat faulty idea of the shape of the adult cavity. He, however, mentions some very essential conditions in his descriptions of the adult sinus. His consideration of the cavity is very brief and many important factors are omitted. His report of the case through which his attention was called to this cavity, is unique and interesting.

The adult sinus maxillaris, as we know it, is the large cavity within the body of the maxilla. It is the largest of the sinus paranasales, save in exceptional cases, when it is comparatively small and may be exceeded in size by the sinus frontalis and the

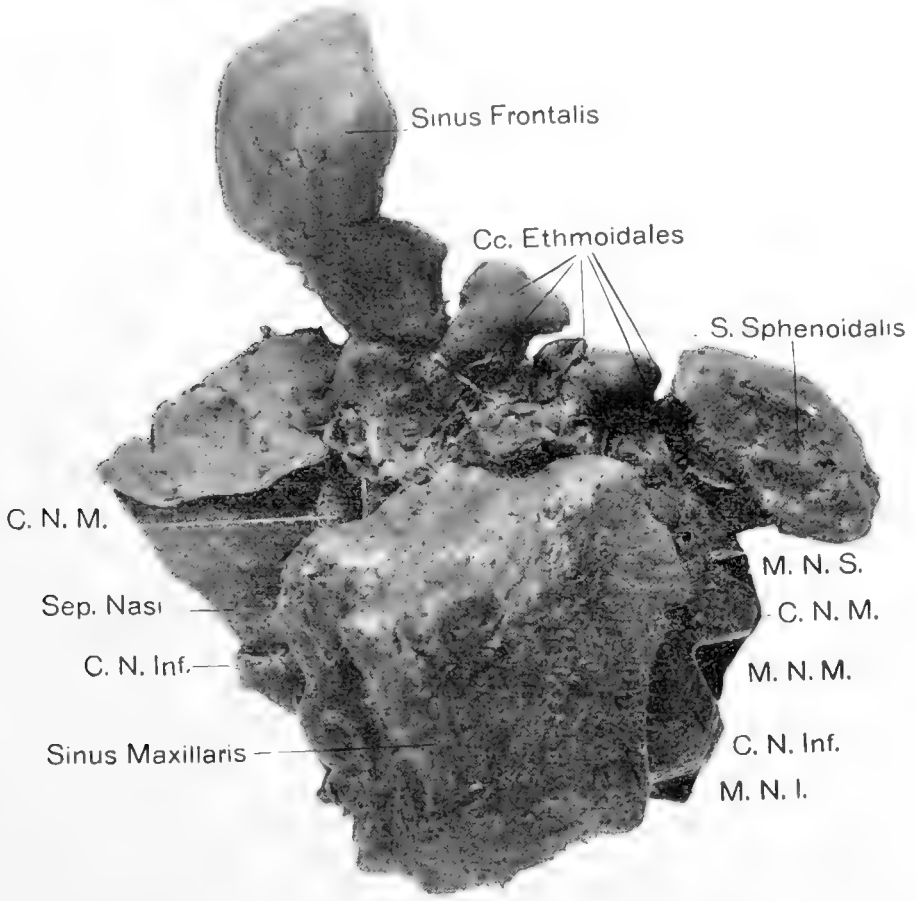


FIG. 7 (0.9). Photograph of a dissection of the sinus paranasales of the left side. The mucous membrane is shown, the bony walls have been dissected away after first hardening the subject in formalin.

C. N. M., C.N. Inf., = conchæ nasales, media et inferior; **Sep. Nasi,** = septum nasi; **Cc. Ethmoidales,** = cellulae ethmoidales; **S. Sphenoidalis,** = sinus sphenoidalis; **M. N. S., M. N. M., M. N. I.,** = meatus nasi, superior, medius, et inferior, respectively.

sinus sphenoidalis. It lies lateral to the cavum nasi and resembles in shape a three sided pyramid (fig. 7). It follows in the main the shape of the body of the maxilla; and may be described as having a roof, a floor, and three walls. The walls of the sinus vary in thickness, usually from 5 to 8 mm.; but they may be

reduced to a papery delicacy. The base or median wall is directed towards the cavum nasi, and the apex of the sinus extends into the root of the processus zygomaticus of the maxilla. It may even extend into the maxillary border of the zygomatic bone; thus extending the recessus zygomaticus of the sinus maxillaris.

The ventral wall of the cavity corresponds to the anterior or facial surface of the maxilla, and looks ventrolaterally. Part of this wall is at times greatly approximated to the dorsal wall and base of the sinus, due to a very prominent fossa canina. Occasionally the whole ventral wall bulges markedly into the cavity of the sinus.

The dorsal wall of the sinus corresponds to the infratemporal surface of the maxilla. It is a thin plate of bone, also forming the ventral boundary of the infratemporal and the pterygopalatine fossæ. This wall is usually the thickest of the sinus walls—it is, however, occasionally extremely thin (the processus alveolaris being recognized as the floor of the cavity and not as a wall).

The base or median wall is directed towards the cavum nasi. It presents a very irregular orifice—hiatus maxillaris, in the disarticulated bone. In the articulated skull this opening is partly filled in by the pars perpendicularis of the palate bone, the processus uncinatus of the ethmoid bone, the processus maxillaris of the inferior nasal concha, and a portion of the lacrimal bone. In the undissected state this irregular aperture formed by these bones is rounded by mucous membrane, which is continued into the sinus maxillaris from the cavum nasi. This rounded opening—ostium maxillare, may be duplicated; and such duplication must not be confused with the ostium maxillare accessorium, which is a direct passageway between the sinus and the cavum nasi. The ostium or ostia maxillaria establish a communication between the sinus and the infundibulum ethmoidale. The medial wall immediately inferior to the attachment of the concha nasalis inferior is very thin and is easily punctured in this region. This wall also forms the lateral boundary of the cavum nasi and often markedly encroaches upon the cavity of the sinus maxillaris, which greatly influences its size.

The roof of the sinus maxillaris is a very thin plate of bone, at times of a papery delicacy. It also forms the floor of the orbit and the orbital surface of the maxilla. It is often modeled by a ridge formed by the infraorbital canal. In some cases the ridge is replaced by a groove which is covered over with the mucous membrane of the cavity. At times the roof of the sinus is partially divided into two plates separated by air cells. Occasionally the palate bone aids in forming the roof.

The floor of the sinus is formed by the processus alveolaris of the maxilla. It is by far the thickest of the osseous boundaries of the cavity—the thickness of the floor depending upon the degree of hollowing out of the process. In cases where the hollowing out has been carried far, the floor of the sinus will bear an important relation to some of the teeth and their sockets. The floor of the sinus may be thrown into irregular elevations by the fangs of the teeth—this depending upon the thickness of the layer of spongy bone. This layer varies in thickness in different skulls and there may be considerable asymmetry on the two sides of the same skull. The relation of the teeth will be considered in a subsequent paragraph.

THE RELATION OF THE SINUS FLOOR TO THE NASAL FLOOR.

The relation of the floor of the sinus maxillaris to the floor of the nose depends largely upon the degree of hollowing out of the processus alveolaris of the maxilla. The degree of arching of the palatum durum—thereby affecting the floor of the nose, has also some bearing on this relation. When the layer of spongy bone is thin, i. e., the processus alveolaris of the maxilla markedly hollowed out, the floor of the sinus is at a level inferior to the nasal floor. On the other hand, when the processus alveolaris is comparatively thick, the floor of the nose is inferior to the sinus floor. Occasionally both floors are in the same plane (figs. 8, 9 and 11). When the anterior surface of the maxilla and the lateral wall of the nose are markedly bulging towards the sinus maxillaris, the floor of the nose is, as a rule, *inferior to the floor of the sinus*. It, however, remains that the majority of sinuses

have their floors, at varying distances, inferior to the floor of the nose. Sixty adult specimens were examined to ascertain this relation, with results as appended:

NUMBER EXAMINED	SINUS FLOOR INFERIOR	NOSE FLOOR INFERIOR	SAME LEVEL
60	39	12	9

The difference in levels of these two floors, when not in the same plane, varies from one-half to 10 mm. C. Resschreiter says that it is a male characteristic to find the sinus floor at an

FIG. 8 (×. 385). Photograph of a dissection of the sinus maxillaris and frontalis from a ventral view.

S. Fron., S. Max., = sinus frontalis et maxillaris, respectively.

inferior level to the nasal floor. I have, however, been unable to verify this statement, and give the following as typical of my findings:

NUMBER EXAMINED	SINUS FLOOR INFERIOR	NOSE FLOOR INFERIOR	SAME LEVEL
12 (female)	9	2	1

RELATION OF THE SINUS MAXILLARIS TO THE TEETH.

Since the sinus maxillaris varies greatly in size in different skulls, and on the two sides of the same skull, it at once becomes apparent that the relations of the teeth to the sinus cannot be constant. As stated before, the layer of spongy bone between

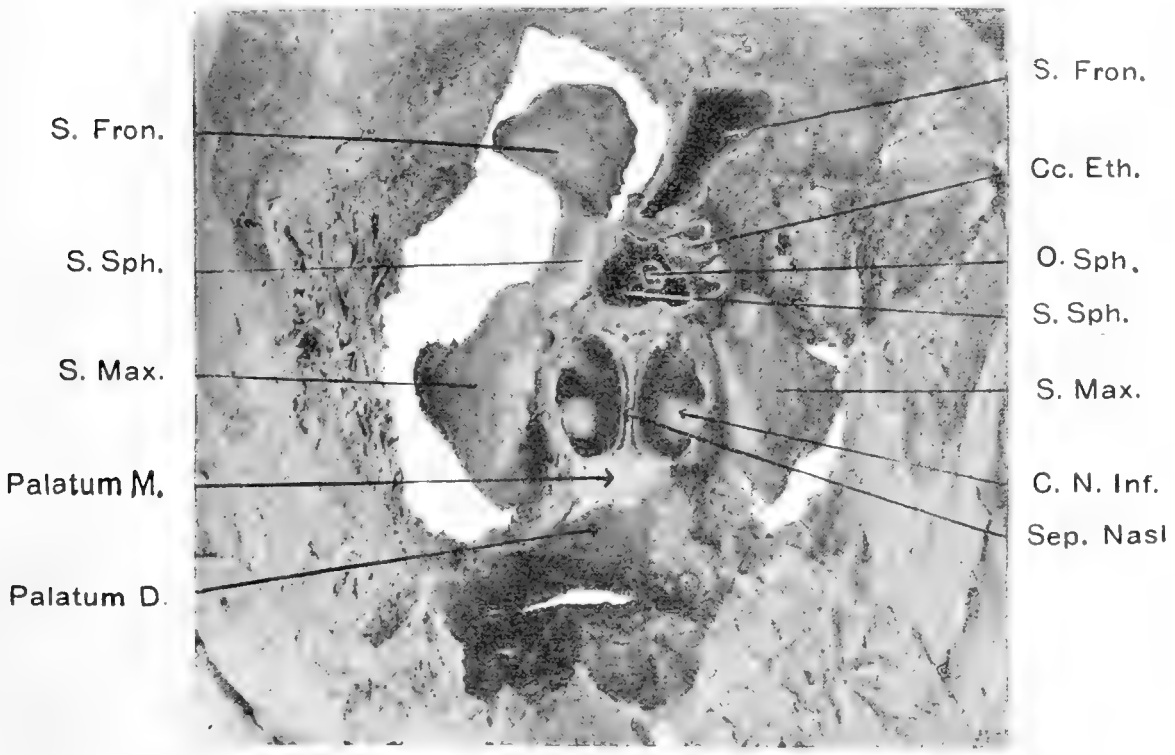


FIG. 9 (× .64). Photograph of a dissection of the sinus paranasales from a dorsal view. The mucous membrane of the cavities is shown, the bony walls have been dissected away. The sinus frontalis and sphenoidalis, and the cellulæ ethmoidales of the right side have been opened.

S. Fron., **S. Sph.**, **S. Max.**, = sinus frontalis, sphenoidalis, and maxillaris, respectively; **Palatum M.**, **Palatum D.**, = palatum molle and palatum durum, respectively; **Cc. Eth.**, = cellulæ ethmoidales; **O. Sph.**, = ostium sphenoidale; **C. N. Inf.**, = concha nasalis inferior; **Sep. Nasi.**, = septum nasi.

the roots of the teeth and the floor of the sinus varies in thickness in different skulls, and the asymmetry on the two sides of the same skull is at times marked. When this layer of spongy bone is comparatively thin the projecting tooth fangs form elevations, of a greater or less degree, on the floor of the sinus. These elevations at times aid in recess formation (fig. 16). Direct communi-

cation between the fangs of the teeth and the mucous membrane of the sinus, due to extreme hollowing out of the processus alveolaris of the maxilla, occurs most frequently in the aged (fig. 15). This latter condition does, however, occasionally prevail in the young adult (fig. 16). That very intimate relations frequently exist between the teeth and the sinus maxillaris is a fact that we should be cognizant of, but I find that these *intimate* relations have been somewhat exaggerated by some writers.

Fig. 10 (× .8). Photograph of a frontal section of a child's face aged from 16 to 18 months. Note the infraorbital canal and nerve, and the relation of the sinus maxillaris to the developing teeth. It will be noticed that the sinus maxillaris has developed sufficiently to reach beneath the orbit but that it is medial to the infraorbital canal.

C. Info., = canalis infraorbitalis; S. Max., = sinus maxillaris.

The number of teeth that bear a *direct* relation to the sinus is necessarily inconstant, as stated before. In exceptional cases, when the cavity is very large—especially in the line of the ventrosuperior diagonal, all of the teeth of the *true* maxilla may be in relation with the sinus (fig. 15). It is, however, only an occasional occurrence to have the canine in direct relation with the sinus. In a certain number of cases the first premolar tooth

bears a direct relation to the cavity, and in a slightly larger percentage of cases the second premolar bears a similar relation. *The three most constant teeth, however, in direct relation to the sinus are the three molars.* When the sinus maxillaris is small the first molar must be omitted from the direct relation (figs. 12 to 16).

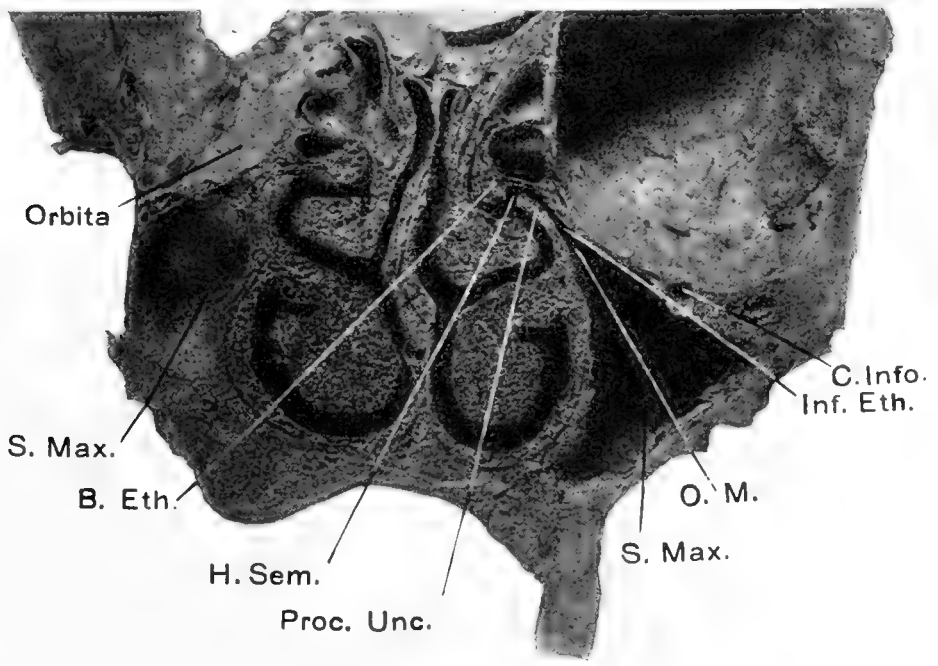


FIG. 11 (× .8). Photograph of a frontal section of an adult's face in the region of the sinus maxillaris. Note the location of the ostium maxillare and th infundibulum ethmoidale. Although the section is not exactly a frontal one there is nevertheless a marked asymmetry in the size of the two sinus maxillares. There is also quite a difference in the relation of the sinus floor to the nasal floor on the two sides.

S. Max., = sinus maxillaris; **B. Eth.**, = bulla ethmoidalis; **H. Sem.**, = hiatus semilunaris; **Proc. Unc.**, = processus uncinatus; **C. Info.**, = canalis infraorbitalis; **Inf. Eth.**, = infundibulum ethmoidale; **O. M.**, = ostium maxillare.

It is a fairly safe rule to follow that, when the canine fossa and the lateral nasal wall are simultaneously approximated, the canine and premolar teeth do not bear a direct relation to the sinus maxillaris. In such cases a perforator pushed through a premolar-tooth socket might readily enter the lateral nasal wall—even pass through it, passing entirely free of the sinus cavity. Again, if the perforator were pushed through the lateral nasal wall, inferior

Figs. 12 to 17. Photographs of dissections showing variations in the teeth relations of the sinus maxillaris.

Figs. 12, 13 (× .48). Note the short ventrosuperior diagonal of the sinus maxillaris, due to the simultaneous approximation of the ventral wall and the base of the sinus. As a result, the only teeth in *direct* relation to the cavity are the second and third molars.

Fig. 14 (× .456). Note that the premolar teeth are not in direct relation, and that the canine tooth, as in the preceding figures, would certainly be a bad tooth to use in attempting to drain the cavity through the canine socket.

Fig. 15 (× .48). This figure shows to what extreme the body of the maxilla may be hollowed out by the sinus maxillaris. Note the very delicate walls of the cavity, especially the shell-like alveolar process. Due to the extensive recessus alveolaris the "remaining tooth" projects into the lumen of the cavity and is merely covered with the mucuous membrane of the sinus.

Fig. 16 (× .552). Although the processus alveolaris is comparatively thick, the second-molar tooth fangs just reach the mucous membrane of the cavity. Note the large ostium maxillare accessorium.

Fig. 17 (× 1.28). Showing the relations of the developing teeth to the sinus maxillaris, of a child aged from 18 to 20 months. Note the position of the *Anlagen* of the permanent teeth.

S. Max., = sinus maxillaris; **C. P.**, = crescentic projection; **D. p. r.**, = dentes permanentes rudimentii; **Sep. Nasi.**, = septum nasi; **F. Info.**, = foramen infra-orbitale; **D. d.**, = dentes decidui.

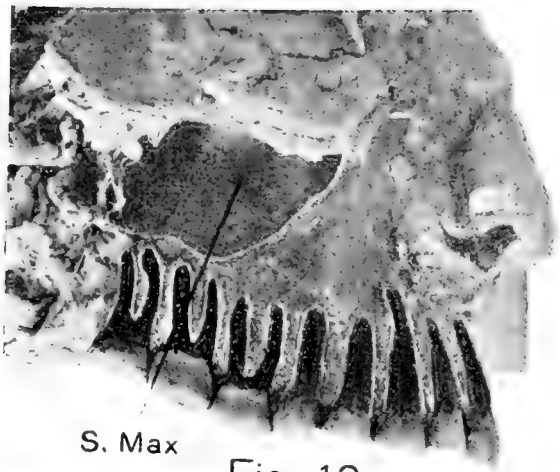


Fig. 12

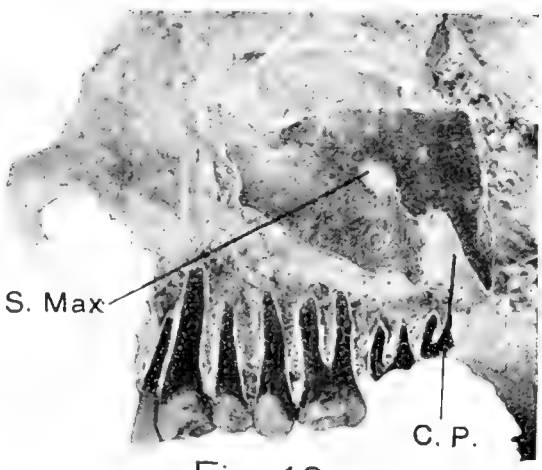


Fig. 13

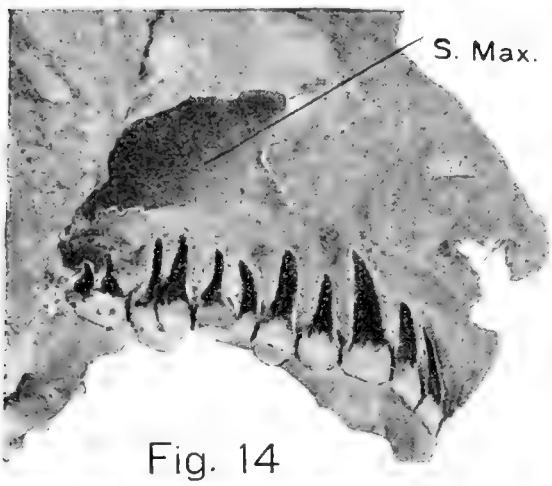


Fig. 14

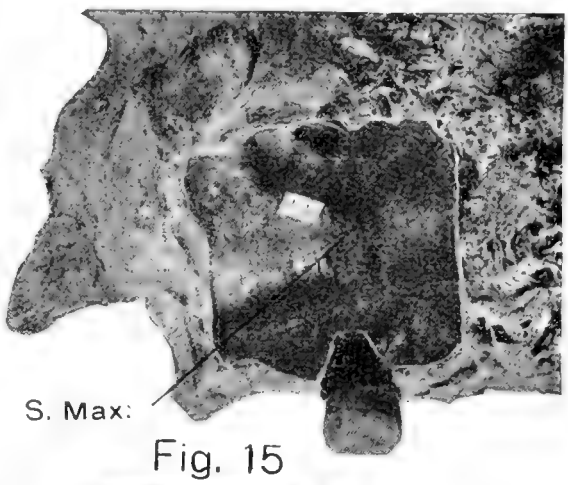


Fig. 15

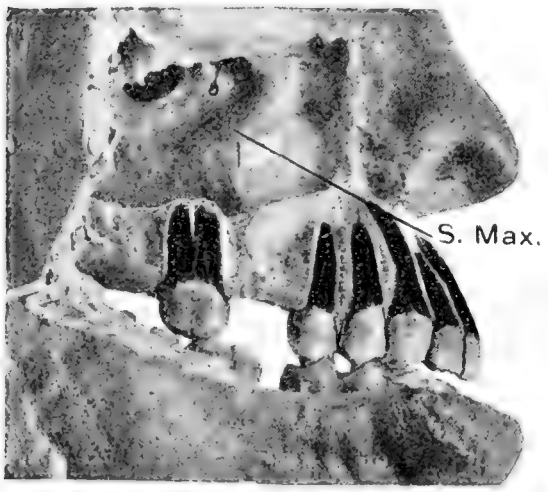


Fig. 16

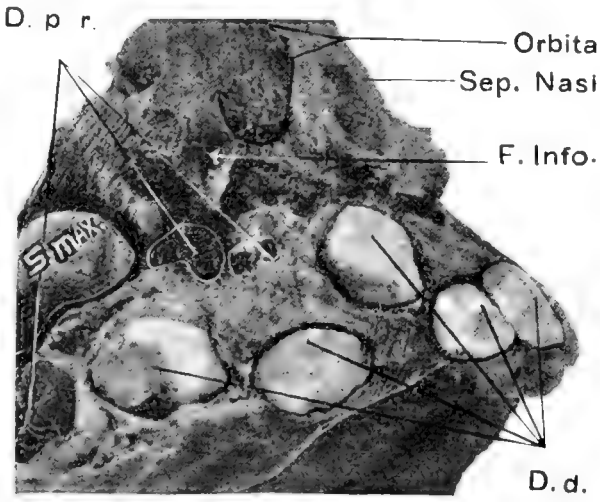


Fig. 17

to the concha nasalis inferior, the instrument could readily be pushed through the soft structures of the cheek, unless the point were directed well superodorsally.

RIDGES, CRESCENTIC PROJECTIONS, AND SEPTA ON THE SINUS WALLS.

It is important to note how frequently the walls of the sinus are found uneven. These irregularities may consist of *mere ridges* or of different sized *crescentic projections.* The crescentic projections have been reported occasionally replaced by septa which completely divide the sinus into two cavities, each having its independent opening into the nasal fossa, but not communicating with each other (fig. 22). The smaller ridges are of little consequence and may be omitted from further consideration. The larger ridges and crescentic projections, on the other hand, tend to form pockets and recesses, of varying depths, within the cavity. The septa, when they exist, may be placed either superoinferiorly or ventrodorsally; thus forming either ventral and dorsal, or inferior and superior compartments, respectively. According to Zuckerkandl's findings, the superior and dorsal cavities communicate with the meatus nasi superior, and the ventral and inferior cavities with the infundibulum ethmoidale. Bruhl found the inferior compartment communicating with the meatus nasi inferior. Gruber found a complete division of the sinus maxillaris in 2.5 per cent of cases.

In the material used for this paper no sinus was found showing division into two distinctly separate compartments, but the specimens repeatedly showed crescentic projections and ridges which formed pockets of a greater or less depth (figs. 18 to 23).

Sixty sinuses were examined to cover this phrase of the work, with results as follows:

NUMBER EXAMINED	RIDGES OR CRESCENTIC PROJECTIONS	SINUS WALLS EVEN
60	29	31

It must be borne in mind that, in the 29 positive sinuses, quite a number of them showed mere ridges—the latter will be omitted from further study. The remaining number of the

positive group fall, however, into a very important class of specimens. That these crescentic projections offer, at times, *almost insuperable obstruction* in attempting to drain fluid from the sinus through an opening either in the processus alveolaris, or in the meatus nasi inferior, is a fact that we should be cognizant of. This was repeatedly demonstrated by first filling the sinus with a liquid, then making an opening at some point on the processus alveolaris; thus draining out what would come away. If some of the fluid was retained—allowing for adherence to mucous membrane—the facial or anterior surface of the maxilla was removed to find where the remaining fluid was lodged. As a rule the portion of fluid was retained by a recess or recesses on one or more of the sinus walls. At other times a second and even a third opening was made, either through the alveolar border or through the meatus nasi inferior, before the remaining fluid would come away. If after repeated attempts the fluid could not be located, the ventral wall of the cavity was removed to ascertain the reason for its retention, and the fact was thus demonstrated that *repeated punctures*, in *some* cases, would not reach all of the recesses.

Just what these recesses mean in all cases is difficult to say. Some of them are of course formed by elevations caused by tooth fangs, but these as a rule are of minor importance and only occasionally form deep recesses. Others are formed by projections of mucous membrane, which may or may not be caused by crescentic bone projections. Where complete septa exist, the sinus maxillaris very likely developed from two primary pouches. In some cases the intervening wall may have disappeared in part, thus leaving the larger crescentic projections which occasionally are found in the adult sinus. A double pouching of the primitive sinus maxillaris was mentioned in a previous paragraph on the development of the cavity. Unequal resorption of the bone during the growth of the sinus is doubtless a cause for some projections occurring on the walls of the cavity.

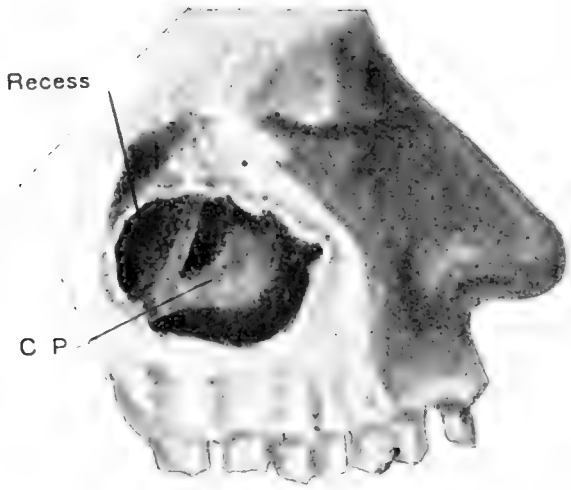


Fig 18

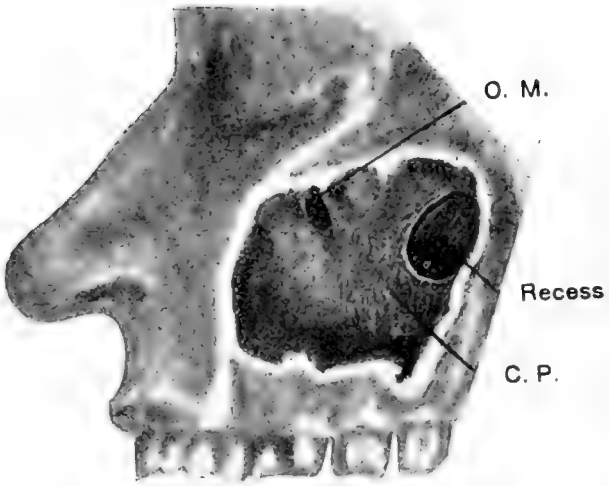


Fig. 19

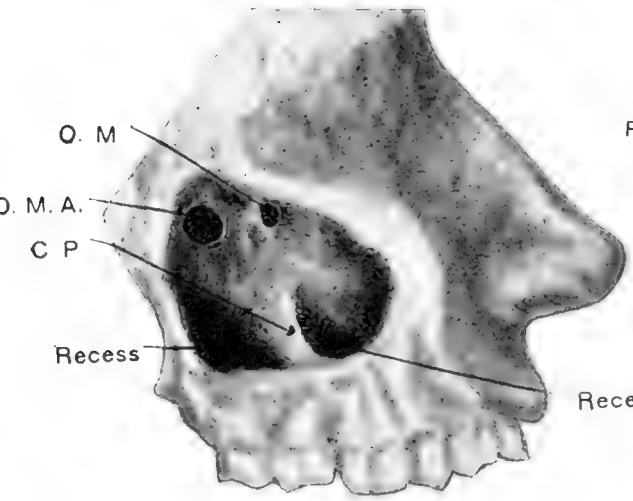


Fig 20

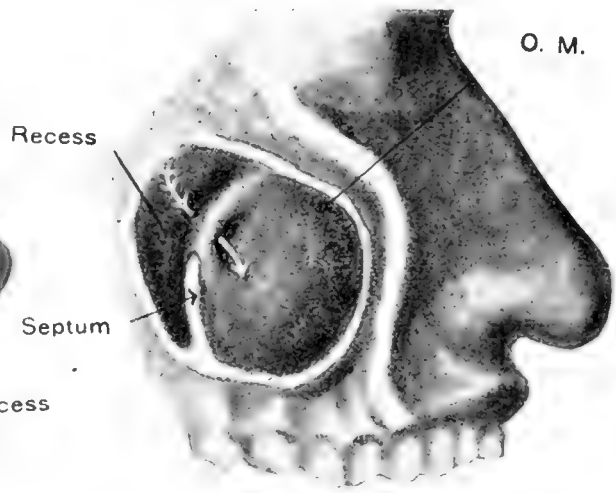


Fig. 21

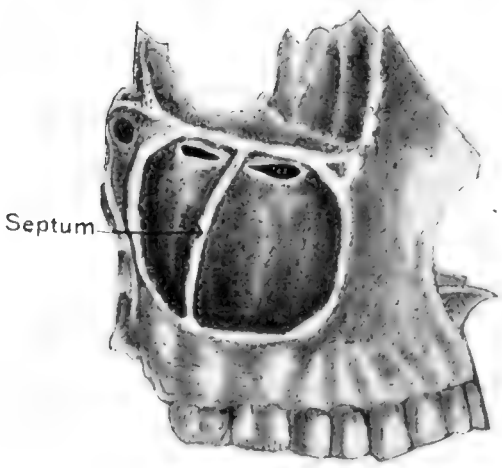


Fig. 22

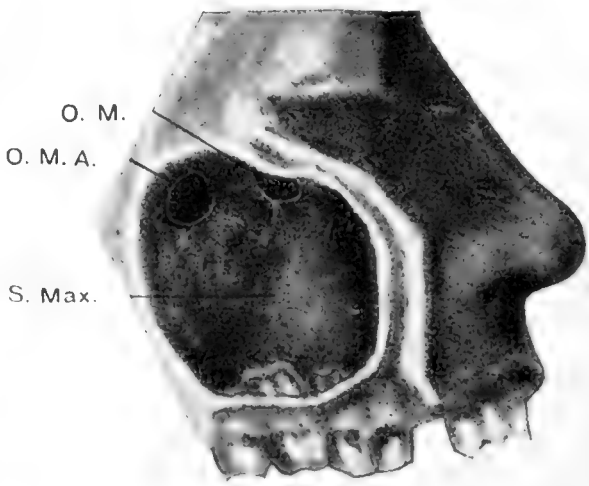


Fig. 23

FIGS. 18 to 23. Drawings of specimens showing septa and crescentic projections on the walls of the sinus maxillaris. Note how these projections form recesses within the cavity. (Fig. 22 is modified from E. Zuckerkandl, Normale und pathologische Anatomie der Nasenhöhle und ihrer pneumatischen Anhänge).

C. P., = crescentic projection; **O. M.**, = ostium maxillare; **O. M. A.**, = ostium maxillare accessorium; **S. Max.**, =sinus maxillaris.

THE SIZE OF THE SINUS MAXILLARIS.

The sinus maxillaris varies greatly in size in different individuals. There may also exist considerable asymmetry on the two sides of the same individual. The statement that *all old* people have large sinuses is very fallacious, as is also the statement that all *females* have smaller sinuses than males (tables A, B, C.)

The investigations of Zuckerkandl have shown that enlargement of the sinus maxillaris may be produced by:

a. Hollowing out of the processus alveolaris of the maxilla (recessus alveolaris);

b. Excavation of the floor of the nasal fossa by a pushing of the recessus alveolaris between the plates of the palatum durum (recessus palatinus);

c. Extension of the sinus maxillaris into the processus frontalis of the maxilla (recessus infraorbitalis);

d. Hollowing out of the processus zygomaticus of the maxilla (recessus zygomaticus);

e. Extension to, and appropriation of an air cell within the processus orbitalis of the palate bone;

To these should be added, according to my findings:

f. Extreme hollowing out of the body of the maxilla in all directions, thus causing the sinus walls to be thin and the recesses all markedly developed;

g. The rarer condition when the lateral nasal wall is bulging towards the cavum nasi;

h. The extension of the recessus zygomaticus of the sinus maxillaris into the maxillary border of the zygomatic bone.

Zuckerkandl has found that the sinus may be made smaller, on the other hand, by:

a. Deficient absorption of the cancellated bone on the floor of the sinus;

b. Encroachment of the ventral wall of the cavity;

c. A deep fossa canina;

d. Thick sinus walls;

e. Excessive lateral bulging of the nasal wall;

f. A combination of the above conditions;

g. Imperfect dentition.

The thickness of the sinus walls varies from 5 to 8 mm. and down to that of a papery delicacy. The statement that all large cavities have *thin* walls and small cavities *invariably thick* walls does not hold in all cases. The smallest sinus measured in this series had the thinnest walls—of a papery delicacy. The smallness of this cavity was in part due to the marked simultaneous approximation of the ventral and medial walls.

The size of the sinus maxillaris is best determined by a series of measurements, viz:

1. Dorsosuperior diagonal (D. S. D.)
2. Ventrosuperior diagonal (V. S. D.)
3. Superoinferior (S. I.)
4. Ventrodorsal (V. D.)
5. Mediolateral (M. L.)

These several measurements are determined thus (fig. 24):

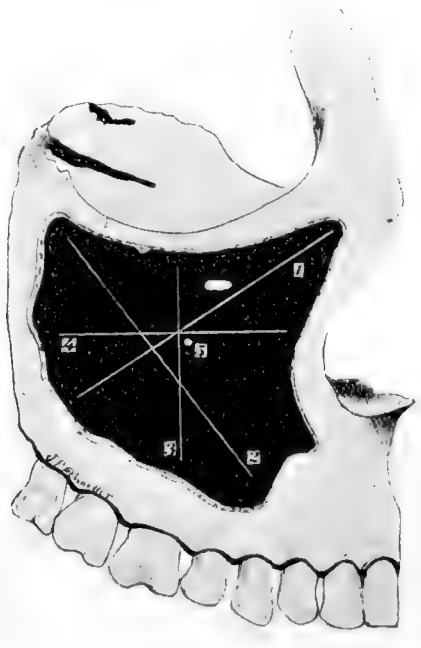


Fig. 24. Schematic drawing of the right maxilla. The ventrolateral wall of the sinus maxillaris has been removed, thus exposing the base or median wall of the cavity. The lines drawn on the base indicate the position of the several measurements.

1, Dorsosuperior diagonal; 2, Ventrosuperior diagonal; 3, Superoinferior; 4, Ventrodorsal; 5, Mediolateral.

1. The dorsosuperior diagonal, from the most dorsal and lateral part of the sinus floor diagonally across the base or median wall of the sinus, to the most medial and superior part of the recessus infraorbitalis;

2. The ventrosuperior diagonal, from the most ventral and medial part of the recessus alveolaris diagonally across the base of the sinus, to the most lateral and superior point of the cavity;

3. The superoinferior, from the roof or infraorbital wall of the sinus, to the sinus floor (always using uniform points);

4. The ventrodorsal, from the most ventral point of the cavity midway between the roof and the floor, to the dorsal wall;

5. The mediolateral, from the base midway between its most ventral and dorsal points, to the processus zygomaticus of the maxilla (in some cases this extends into the maxillary border of the zygomatic bone due to the extension of the recessus zygomaticus of the sinus maxillaris into this bone).

The ventrodorsal distance is especially affected by the degree of approximation of the ventral wall of the sinus; the superoinferior by the degree of hollowing out of the processus alveolaris of the maxilla; the mediolateral by the degree of encroachment of the lateral nasal wall; the ventrosuperior diagonal by the extent of the recessus alveolaris; and the dorsosuperior diagonal by the extent of the recessus infraorbitalis. Of course there are other contributing factors to shorten or lengthen these distances, but these are the primary factors especially affecting the several measurements.

In order that the measurements of the sinus maxillaris may be of most value, it is necessary to compare the two sinuses of the same individual, to compare them with the respective sinuses of another individual; also to consider the age and the sex.

A careful examination of the following tables (A, B, C) will show conclusively that the sinus maxillaris has a rather wide range of variation. These tables also show that in the adult, age does not have much bearing on the size of the cavity. A reference to table C will show that the *smallest* cavity is that of an old man, aged 70 years; while the *largest* cavity is also that of an old man, aged 77 years. This same table shows that the cavity of a young adult, aged 21 years is a close second to the

largest sinus found in the whole series. Although the cavity in the male averages slightly larger than that of the female, a reference to table C will show that sex affects the size of the sinus but slightly.

The following may be given as average measurements of the adult sinus maxillaris, based on the measurements of 90 specimens:

		mm.
1	Dorsosuperior diagonal	38
2	Ventrosuperior diagonal	38.5
3	Superoinferior	33
4	Ventrodorsal	34
5	Mediolateral	23

Due to the great differences in the several measurements, the capacity of the sinus, in different individuals, must also differ. The range in capacity, of the sinuses studied to ascertain this fact, was from 9.5 cc. to 20 cc., with an average of 14.75 cc.

The tables A, B and C show the range of measurements.

The conditions which produce these varied differences in the dimensions of the sinus maxillaris may be readily ascertained. Take for example the following two conditions which show a marked difference in the mediolateral plane and yet the other measurements are inversed:

No.	V. D.	M. L.	S. I.	D. S. D.	V. S. D.
	mm.	mm.	mm.	mm.	mm.
1	30	18	40	41	41
2	35	35	35	40	30

In case number 1 the lateral nasal wall was markedly bulging towards the sinus. In consequence of this encroachment, the mediolateral distance was greatly lessened. In case 2 the recessus alveolaris was poorly developed, hence the short ventrosuperior diagonal in comparison with the respective measurement in case number 1. These cases show that even though a sinus may greatly exceed another in one of its measurements, it may be exceeded in size in its other planes.

Again there may be a great difference in the ventrodorsal distance. This means a marked inpushing of the ventral wall of the

TABLE A.

NUMBER	SEX	AGE	SIDE	VENTRO-DORSAL	MEDIO-LATERAL	SUPERO-INFERIOR	DORSO-SUPERIOR DIAGONAL	VENTRO-SUPERIOR DIAGONAL
				mm.	*mm.*	*mm.*	*mm.*	*mm.*
1	M	54	right	26	15	20	30	26
			left	30	16	22	32	26
2	M	68	right	40	22	50	50	50
			left	35	24	35	45	50
3	M	36	right	32	32	40	40	38
			left	30	18	40	41	41
4	M	65	right	30	15	30	33	30
			left	25	15	25	35	36
5	M	55	right	40	25	40	45	45
			left	40	22	38	36	45
6	M	57	right	40	21	32	50	38
			left	32	25	30	32	43
7	M	71	right	35	22	45	45	40
			left	40	18	35	40	45
8	M	59	right	40	22	33	45	45
			left	40	35	40	50	45
9	M	79	right	30	30	35	41	40
			left	43	20	30	41	37
10	M	55	right	31	24	30	30	38
			left	32	25	35	40	40

TABLE B.

NUMBER	SEX	AGE	SIDE	VENTRO-DORSAL	MEDIO-LATERAL	SUPERO-INFERIOR	DORSO-SUPERIOR DIAGONAL	VENTRO-SUPERIOR DIAGONAL
				mm.	*mm.*	*mm.*	*mm.*	*mm.*
1	F	68	right	35	35	35	40	30
			left	40	16	30	43	36
2	F	52	right	35	21	30	40	40
			left	35	24	28	38	45
3	F	53	right	40	25	30	60	42
			left	33	30	45	45	46
4	F	47	right	36	26	25	37	37
			left	37	28	35	35	37
5	F	73	right	33	24	31	38	40
			left	37	24	37	30	42
6	F	50	right	33	17	30	35	40
			left	33	22	33	38	34
7	F	35	right	30	18	32	30	35
			left	30	21	30	30	40
8	F	39	right	34	25	33	32	35
			left	33	22	33	32	24
9	F	72	right	38	25	38	32	34
			left	35	23	38	33	35
10	F	52	right	35	21	30	40	36
			left	35	21	32	38	35

TABLE C.

NUMBER	SEX	AGE	SIDE	VENTRO-DORSAL	MEDIO-LATERAL	SUPEBO-INFERIOR	DOSRO-SUPERIOR DIAGONAL	VENTRO-SUPERIOR DIAGONAL
				mm.	*mm.*	*mm.*	*mm.*	*mm.*
1	M	70	right	15	12	21	21	18
2	M	70	left	16	12	21	21	20
3	M	35	left	22	20	30	31	25
4	M	54	left	25	15	22	32	27
5	F	54	right	26	15	20	30	26
6	M	60	left	30	20	22	38	25
7	F	52	right	35	25	30	37	38
8	M	59	left	40	22	32	45	45
9	M	21	right	46	33	26	50	50
10	M	77	left	47	40	50	57	60

sinus, on the one hand, and a shallow fossa canina with a lessened encroachment on the other hand. Thus:

No.	V. D.	M. L.	S. I.	D. S. D.	V. S. D.
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
1	25	15	25	35	36
2	43	20	30	41	37

If the body of the maxilla is hollowed out to a marked degree in all directions the measurements will be correspondingly lengthened. When this hollowing out has not been carried far, and when associated with some of the above mentioned conditions, the measurements will be markedly lessened. Thus:

No.	V. D.	M. L.	S. I.	D. S. D.	V. S. D.
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
1	47	40	50	57	60
2	16	12	21	21	20

These few examples show how anatomical conditions will affect the measurements of the sinus maxillaris. It, therefore, appears reasonable that, by examination of the anterior surface of the maxilla and the lateral nasal wall, the size of the sinus may be approximately determined and the teeth relations judged. It does, however, not necessarily follow, because the ventral and

median walls of the sinus are closely approximated, that the sinus capacity is markedly lessened, These sinuses may have marked infraorbital recesses and the processus alveolaris may be hollowed out towards its dorsal termination. In this manner compensation may be made for the marked bulging toward the cavity of the ventral and median walls of the sinus. It, however, remains that in the vast majority of cases, where these walls are simultaneously bulging into the cavity, the sinus is correspondingly reduced in size and the canine and premolar teeth not in *direct* relation to the sinus.

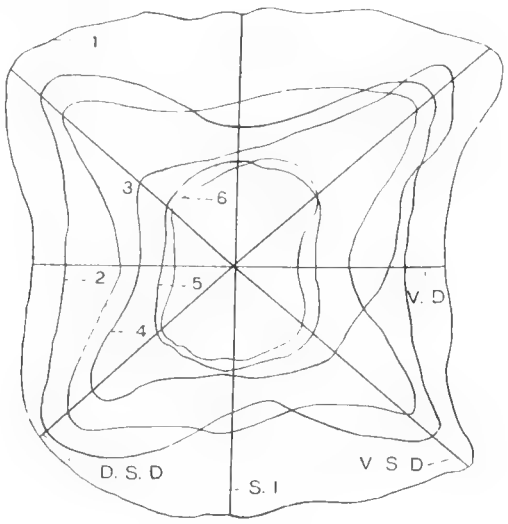


Fig. 25 (× 1). Composite chart showing how anatomical variations in the extent of the recesses and the approximation of the walls of the sinus maxillaris affect the shape and size of its median wall or base.

1 to 6, = outlines of different bases; **V. D., S. I., V. S. D.,D.S. D.,** = ventrodorsal, superoinferior, ventrosuperior diagonal, and dorsosuperior diagonal, respectively.

These variations in the approximation of the sinus walls, and the great difference in the extent of the various recesses, have a marked effect on the shape of the base of the cavity. A reference to figure 25 will show various shapes and sizes. Note especially case 4 in which the ventrosuperior diagonal is very short, and the dorsosuperior, because of a marked infraorbital recess, comparatively long. The great difference in the two diagonals produces a peculiarly shaped base.

THE OSTIUM MAXILLARE.

When considering the embryology of the lateral nasal wall it will be remembered that the primitive maxiliary pouch had certain relations of importance. These structures were the processus uncinatus, the infundibulum ethmoidale, the hiatus semilunaris, and the bulla ethmoidalis. The location of the ostium maxillare, of the adult, corresponds to the place of the primitive maxillary pouch. This pouch gradually develops into the pyramidal cavity of the adult, leaving the place of communication with the infundibulum ethmoidale at the point of primary evagination. It is, therefore, quite evident that these structures which in the embryo bore so close a relation to the Anlage of the sinus maxillaris, must now bear even more important relations to the ostium maxillare.

On raising or removing the middle nasal concha, in the adult, a rounded elevation—the bulla ethmoidalis, is seen. This structure is directed inferiorly and ventrally. Immediately beneath it is the well defined curved margin of the processus uncinatus of the ethmoid bone. Between these structures there is a narrow slit or semilunar cleft—the hiatus semilunaris, which is from 15 to 20 mm. long. This is an important opening, for it serves as the communication between the meatus nasi medius and the gutter-like groove (infundibulum ethmoidale) formed by these structures. The bulla ethmoidalis varies considerably in size. At times it is feebly developed and again it may assume comparatively large proportions. The size of the bulla greatly influences the width of the semilunar cleft or hiatus semilunaris. The bulla may be so large that its convexity comes in direct contact with the free margin of the processus uncinatus of the ethmoid bone. In other cases the hiatus semilunaris may be of considerable width.

It is easy to conclude what effect these conditions will have on the ostium maxillare *directly*, and on the sinus maxillaris *indirectly*. In one case the cleft of communication between the ostium maxillare and the meatus nasi medius is practically shut off, while in the other case a freer communication exists. It must be remembered that, even though the bulla touches the free margin

of the processus uncinatus—thus greatly narrowing the hiatus semilunaris, the infundibulum ethmoidale may be of average dimensions. This is an important fact, and must always be borne in mind when considering the fronto-maxillary relations.

The processus uncinatus with its covering of mucous membrane projects inferiorly and dorsally. By its free superior border it forms the inferior boundary of the hiatus semilunaris. This process frequently terminates dorsally in what may be termed two roots; the inferior one passes towards the superior edge of the concha nasalis inferior, while the superior root curves superiorly behind the dorsal termination of the bulla ethmoidalis (figs. 28 and 29). Such a condition, as the latter, causes the infundibulum ethmoidale to end dorsally in a pocket. This fact is of extreme importance because the pocket is so situated that it will direct any fluid coming to the *dorsal end* of the infundibulum ethmoidale into the sinus maxillaris, via the ostium maxillare which is in the immediate location.

The infundibulum ethmoidale is a groove or gutter situated upon the lateral nasal wall. It is bounded superiorly by the inferior surface of the bulla ethmoidalis throughout the greater part of its extent, save ventrally and superiorly where the bulla is replaced by some anterior ethmoidal cells. The inferior and medial boundary of the groove is formed by the lateral surface of the processus uncinatus. This groove communicates with the meatus nasi medius through the hiatus semilunaris. The infundibulum may end, as stated above, in a pocket; or may loose its depth gradually and be lost in the meatus nasi medius (figs. 28, 29, 30). The superior and ventral end of the infundibulum may terminate blindly without dilatation, or in an air cell; or may be continuous with the nasofrontal duct. The lateral wall of the infundibulum is formed partly by mucous membrane. The depth of this gutter-like channel, or the distance from the superior border of the processus uncinatus to the floor of the groove, varies from 1 to 12 mm., with approximately an average of 5 mm.

The sinus maxillaris communicates *indirectly* with the meatus nasi medius by means of an opening—the ostium maxillare—which pierces the superior and ventral part of the base of the

cavity to open into the infundibulum ethmoidale, thence via the hiatus semilunaris into the meatus nasi medius. It must be clearly kept in mind that the ostium is located in the superior part of the sinus, and that it opens into infundibulum ethmoidale and not into the hiatus semilunaris as many writers say. The ostium maxillare may be either in the most dependent part of the infundibulum or in the lateral wall of this channel. This opening varies in distance from the hiatus semilunaris from 1 to 12 mm. This distance is dependent upon the width of the processus uncinatus and the resultant depth of the infundibulum ethmoidale at this point.

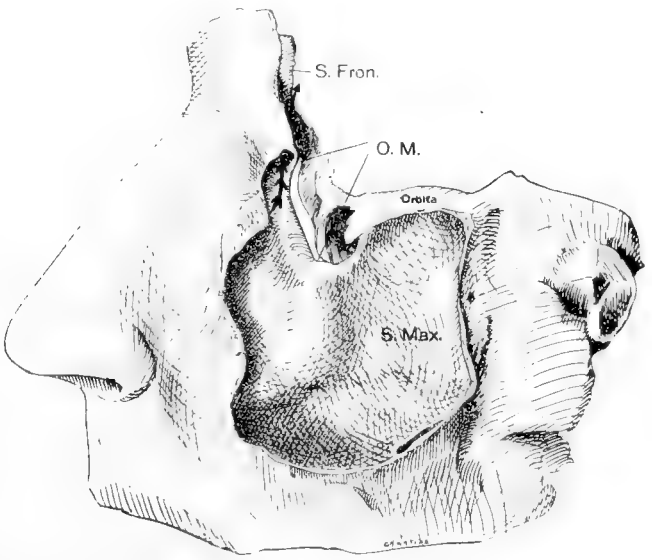


Fig. 26 (× .66). Drawing of a specimen showing a direct communication between the sinus frontalis and maxillaris (indicated by the arrow).

Note the very large slit-like ostium maxillare. The lateral wall of the infundibulum ethmoidale is entirely wanting.

S. Fron., = sinus frontalis; **S. Max.**, = sinus maxillaris; **O. M.**, = ostium maxillare.

The ostium may be round, but as a rule is either oval or elliptical. In my series of 90 cases it has a great range of dimensions; varying from 1 to 20 mm. in length, and from 1 to 6 mm. in width. In some cases where the ostium has reached considerable size it may almost entirely replace the lateral wall of the infundibulum ethmoidale, thus forming a long slit-like communication

between the sinus maxillaris and the infundibulum ethmoidale (fig. 26) (table D, nos. 7 to 12).

The following table gives an idea of the range of dimensions of the ostium maxillæ as found in the series of specimens studied:

TABLE D.

NUMBER	LENGTH *mm.*	WIDTH *mm.*
1	1	1
2	3	3
3	3	2
4	5	3
5	7	4
6	8	3
7	10	6
8	11	4
9	11	6
10	14	3
11	19	3
12	20	3

THE OSTIUM MAXILLARE ACCESSORIUM.

In many cases the sinus maxillaris has an accessory ostium communicating directly with the meatus nasi medius—the ostium maxillare accessorium. This opening is, as a rule, situated in the membranous portion of the lateral wall of the meatus nasi medius a short distance above the superior border of the concha nasalis inferior, at about the junction of its middle and posterior, thirds. In some instances the accessory ostium is placed immediately behind the dorsal termination of the infundibulum ethmoidale (fig. 27). This accessory ostium must not be confused with the duplication of the ostium maxillare, which communicates with the infundibulum ethmoidale.

According to Chiari and Hajek an accessory opening is found in every fifth case in the meatus nasi medius, posterior and inferior to the normal aperture. Giraldès says it is found in 10 per cent of cases, and represents a pathological condition. Zuckerkandl and Kallius report it present in 10 per cent of cases. Turner found it four times in nine dissections.

That this accessory opening occurs more frequently than is generally supposed seems proven by the study of 80 adult speci-

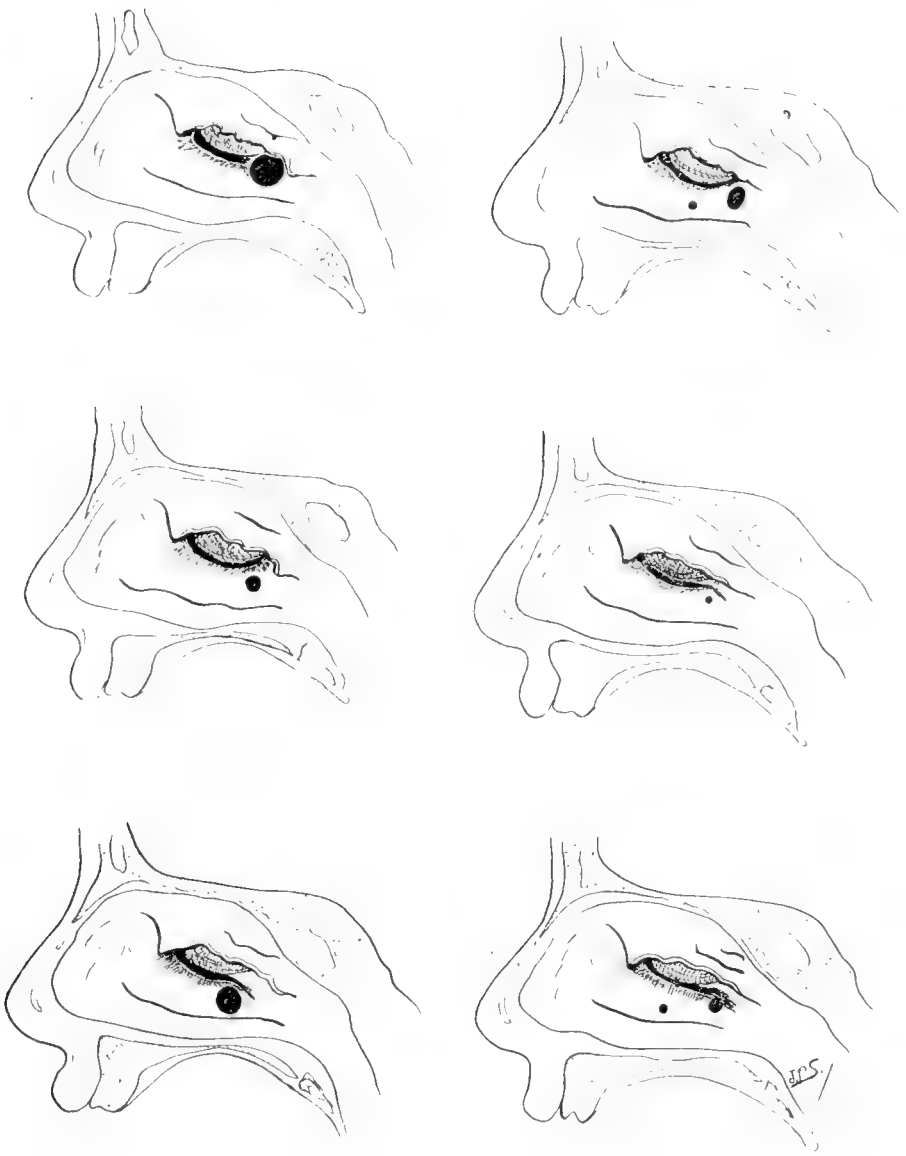


Fig. 27. Diagrams of the lateral nasal wall. The conchæ nasales mediæ have been partially cut away so as to bring to view the underlying structures. Note the positions and varying sizes of the ostium maxillare accessorium in the different diagrams. The accessory ostia are designated by the deep black circles. The upper and lower right hand diagrams show two accessory ostia and the others but one.

mens. *Out of 80 specimens examined 35 showed accessory ostia, or a percentage of 43; while three cases had two accessory ostia, or a percentage of 3.75.* From this it seems that the former figures were much too low. Whether this series had a special run for accessory ostia or whether too few specimens were used in the former reports is of course not known. It may, however, be said that the opening occurs very frequently and that the earlier reports, apparently, placed the percentage of occurrence far too low.

Just what the ostium maxillare accessorium means in all cases is indeed difficult to say. It seems almost incredible that so large a percentage of specimens should have pathological openings. Giraldès bases his claim of a pathological origin on the facts that the accessory ostium is absent in the young individual, and that the mucous membrane becomes thinned out in this locality even though the opening fails to establish itself. Zuckerkandl corroborates the thinning of the mucous membrane in this locality at times, but claims that we have no evidence that it is always a pathological process causing this condition. He says that occasionally the accessory opening is caused by neighboring structures.

Seltenenfalls entsteht ein Ostium maxillare accessorium durch Druck von Seite nachbarlicher Organe; ich habe gesehen, das ein abnorm breiter zugespitzer Hakenfortsatz der Nasenscheidewand an der hinteren Nasenfontanelle eine Durchlocherung veranlasst hatte.

I have not found the accessory ostium present in the fetus and infant. Unfortunately I have been unable to secure a sufficient number of specimens between the ages of 6 and 15 years to draw any conclusions of value on the occurrence of the ostium maxillare accessorium during this period of time. I found the accessory opening occasionally present in the young adult—17 to 20 years. The specimens (adult) studied, ranged in age from 17 to 80 years, with the majority from subjects over 50 years old. That some cases of accessory ostia are of pathological origin is doubtless true, but many cases certainly do not give any evidence of a pathological process. The thinning of the mucous membrane.

of which Giraldès and Zuckerkandl speak, is very evident in many specimens. I, however, believe with Zuckerkandl that we must, in the majority of cases, look elsewhere than to a pathological process for the determining factor in this condition.

In this connection it is important to note that out of the *35 sinus maxillares having accessory ostia, 27 of them had positive relations with the sinus frontalis*; i. e., the infundibulum ethmoidale continuous with the nasofrontal duct. This would indicate that 77 per cent of sinus maxillares having positive fronto-maxillary relations have accessory ostia communicating directly with the meatus nasi medius.

Another explanation for this accessory ostium may be found in the fact that since the sinus maxillaris develops by the growth of the sac and resorption of surrounding bone, its walls have a tendency to become thinned out most at points of least resistance. Such a point is found in the membranous portion of the base of the sinus, where bone is entirely wanting—the usual seat of the accessory opening. The mucous membrane in this position may become thinned out to such an extent, by the growth of the sinus, that an opening is formed; thus establishing the ostium maxillare accessorium.

Since the ostium maxillare opens into the infundibulum ethmoidale, and secondarily by way of the hiatus semilunaris into the meatus nasi medius, it is apparent that the ostium maxillare accessorium, with its more dependent location and direct communication with the meatus nasi medius, is more advantageously placed as a drainage opening for the sinus maxillaris. In some cases the ostium maxillare certainly seems inadequate—due to its *position, relations,* and *size*—to properly drain the sinus. Why then may we not say that this accessory ostium, in some cases, of necessity comes to be formed as a means by which the sinus maxillaris can more readily dispose of accumulated fluid? The process by which this is brought about need not necessarily be termed pathological. Doubtless more information is necessary on this point before we dare draw conclusions.

Of course some specimens present accessory ostia which look decidedly pathological; and as Zuckerkandl points out some are

due to pressure caused by neighboring structures. I hope to study the subject more extensively in the embryo and child to see whether the opening, after all, at times, does not have an embryological significance. Thus far I must agree with Giraldes that the ostium maxillare accessorium does not appear in the embryo and young child.

The accessory ostium varies much in size. In the series I studied the range of measurements was from 1 to 10 mm. long, and from one-half to 10 mm. wide. The opening may be round or elliptical.

The appended table selected from a series of 80 specimens gives the range in size:

TABLE E.

LONG *mm.*	WIDE *mm.*
1	$\frac{1}{2}$
2	1
4	4
6	4
7	5
10	10

THE FRONTO-MAXILLARY RELATIONS.

It is interesting to note that Nathaniel Highmore (1651) recognized the fact that the sinus maxillaris at times receives fluid from other sources. In his description of the cavity (see previous paragraph) he makes brief mention of this important condition.

> Antrum hoc frequentius vàcuum, aliquando muco repletum reperitur, in quod humores a capite per meatum quendam a cavitate illa in osse frontis, et ab osse ethmoeide distillare poterunt.

Although mentioning that fluid from the cavities in the frontal and ethmoid bones occasionally reaches the sinus maxillaris by way of the "meatum," he does not attempt to explain how this is brought about.

Tillaux ('40) found when injecting fluid into the sinus frontalis that some of it passed into the sinus maxillaris, instead of

the whole amount passing into the meatus nasi medius. Cryer ('94, '01, '07), Fillibrown ('96, '97), reported on fronto-maxillary relations. Lothrop's investigations ('98) show that in 47 per cent of cases the infundibulum ethmoidale is continuous with the nasofrontal duct, while 53 per cent show that the infundibulum ethmoidale has no connection with the sinus frontalis. Turner ('01) speaks briefly about the relation, and Wilson ('08) in his paper on the "Variations of the Ostium Frontale" alludes to this important relation. Some clinicians have reported isolated cases where they believed the maxillary trouble secondary to preëxisting frontal trouble; without, however, attempting to explain any anatomical conditions which would justify the clinical conclusions.

In order to secure the fronto-maxillary relations in the specimens at hand, I undertook a series of investigations; including special dissections, filling the sinus frontalis with a fluid to determine the direction of drainage, and the determination of the efficiency of the infundibulum ethmoidale.

It will be remembered that the infundibulum ethmoidale at its superior and ventral termination is either continuous with the nasofrontal duct; or ends blindly without dilation, or in an air cell. The cases where it is continuous with the nasofrontal duct or with the sinus frontalis directly, represent what will be here spoken of as the *positive fronto-maxillary relations.* Where the infundibulum ends blindly or in an air cell, the conditions will be spoken of as *negative fronto-maxilary relations.*

According to the specimens I examined, the sinus frontalis may discharge fluid put into it in one of the following ways:

a. By the nasofrontal duct or the sinus frontalis being continuous with the infundibulum ethmoidale (in some cases there is no nasofrontal duct and the sinus frontalis is directly continuous with the infundibulum ethmoidale) (positive relation) (fig. 28).

b. By the nasofrontal duct communicating directly with the meatus nasi medius (negative relation) (fig. 29).

c. By a combination of the above conditions—in which case the sinus frontalis had two nasofrontal ducts; one continuous

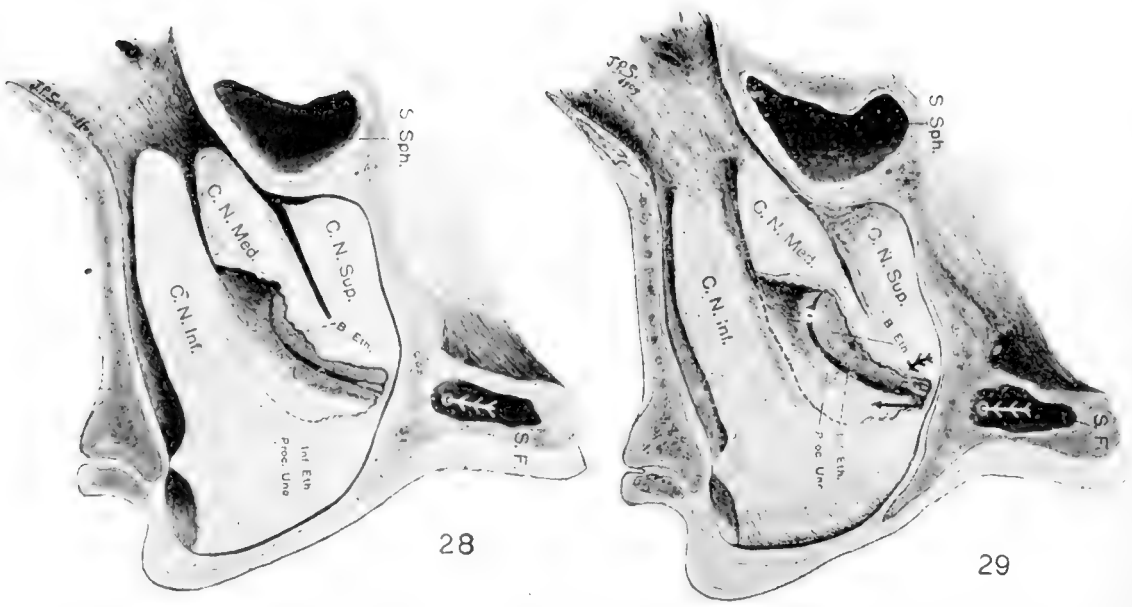


Fig. 28. A semidiagrammatic drawing of the lateral nasal wall showing positive fronto-maxillary relations. Note that the infundibulum ethmoidale is directly continuous with the naso-frontal duct. Note also the superior and lateral curving of the processus uncinatus at its dorsal termination, thus forming a pocket at the dorsal end of the infundibulum ethmoidale. This pocket is so situated that it will direct fluid coming to the *dorsal end* of the infundibulum ethmoidale to the ostium maxillare and into the sinus maxillaris.

The concha nasalis media is in part cut away so as to expose the underlying structures.

Fig. 29. A semidiagrammatic drawing of the lateral nasal wall with the concha nasalis media partially removed. Note that the infundibulum ethmoidale terminates blindly at its superior and ventral end. The nasofrontal duct communicates directly with the meatus nasi medius and not with the infundibulum ethmoidale as in the preceding figure (28). This represents negative fronto-maxillary relations.

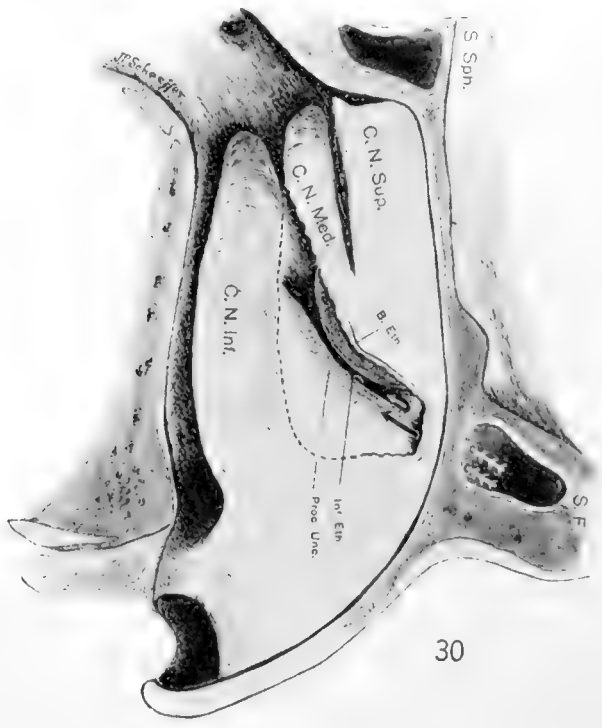


Fig. 30. A semidiagrammatic drawing of the lateral nasal wall showing that the sinus frontalis, in this case, has two nasofrontal ducts, one communicating with the infundibulum ethmoidale and the other with the meatus nasi medius.

Note that the infundibulum ethmoidale terminates at its dorsal extremity in the meatus nasi medius without a pocket formation (compare this condition with figs. 28, 29).

C. N. Sup., C. N. Med., C. N. Inf., = conchæ nasales, superior, media and inferior; **S. Sph.,** = sinus sphenoidalis; **S. F.,** = sinus frontalis; **B. Eth.,** = bulla ethmoidalis; **Inf. Eth.,** = infundibulum ethmoidale; **Proc. Unc.,** = processus uncinatus.

with the infundibulum ethmoidale, and the other communicating directly with the meatus nasi medius (positive and negative relations) (fig. 30).

d. By the nasofrontal duct being continued down to the infundibulum ethmoidale; and in conjunction there being a passage-way between the ventral attachment of the concha nasalis media and the processus uncinatus of the ethmoid bone, to the meatus nasi medius (considered as positive relations).

e. By a direct communication between the sinus maxillaris and the sinus frontalis, by what may be termed the maxillo-frontal duct (direct relation) (fig. 26).

Of the 80 specimens studied to ascertain the fronto-maxillary relations; 45 showed a positive relation, or a percentage of 56.25; 32 a negative relation, or a percentage of 40; 2 a combination of positive and negative, or a percentage of 2.5; 1 a direct communication between the two sinuses, or a percentage of 1.25.

The importance of the above conditions was in each case tested by putting fluid unto the sinus frontalis to determine the course of drainage. It at once became apparent that the specimens falling under classes (*a*) and (*d*) should be classed together as representing positive fronto-maxillary relations. The only difference in the above two conditions is that in class (*a*) all of the fluid put into the sinus frontalis will reach the *superior and ventral* part of the infundibulum ethmoidale; while in class (*d*) some of it will pass directly into the meatus nasi medius, and the remaining portion to the infundibulum ethmoidale.

Class (*b*) will drain fluid from the sinus frontalis directly into the meatus nasi medius. It is, however, important to know that even in these cases some fluid may reach the infundibulum ethmoidale, because of the intimate relations existing between the nasofrontal duct and the superior and ventral end of the infundibulum ethmoidale (fig. 29).

Class (*c*), where the sinus frontalis has two nasofrontal ducts, the drainage is of course partly into the meatus nasi medius and partly into the infundibulum ethmoidale. This class leads to similar results as mentioned above, the only difference being that

the infundibulum does not receive as much fluid in a given time.

Class (*e*) fortunately represents a rare condition. Here the sinus frontalis drains directly into the sinus maxillaris. In the specimen I found with this direct relation there was also a communication between the infundibulum ethmoidale and the sinus frontalis. Cryer, Bryan, and Brophy have reported direct relations between the two sinuses, which I have been able to verify in this one specimen. In cases where the lateral wall of the infundibulum ethmoidale is largely wanting, fluid from the frontal sinus (providing the infundibulum ethmoidale is continuous with the nasofrontal duct) will pass almost directly into the sinus maxillaris, and will, therefore, very closely simulate a direct communication between the two sinuses. A probe passed from the sinus frontalis will, in such cases, also pass into the sinus maxillaris. Doubtless some of these cases have been considered by some clinicians as direct communications, whereas a further dissection would have proved them otherwise (fig. 26).

The question now arises—what happens to the fluid that has reached the superior and ventral end of the infundibulum ethmoidale? In the first place it may be said that the efficiency of the infundibulum ethmoidale, as a carrier of fluid, is in direct ratio to its depth and to the degree of overhanging of the mucous membrane from the free border of the processus uncinatus of the ethmoid bone. In some cases the processus uncinatus is so narrow that the infundibulum ethmoidale has no appreciable depth at its superior end, and in these cases the fluid which has reached it from the frontal region will soon leave the shallow groove after entering it—at least a goodly portion of it. In other cases the processus uncinatus is broad, and the resultant infundibulum ethmoidale *deep* and *channel-like*. It must also be recalled that in a previous paragraph mention was made of the fact that frequently the infundibulum ethmoidale ends dorsally in a *pocket*, so situated that it will direct the flow of fluid coming to the dorsal end of the infundibulum ethmoidale into the ostium maxillare—thence into the sinus maxillaris (the ostium maxillare being patent) (figs. 28, 29).

We have, therefore, a gutter-like channel, of varying depth and efficiency, communicating between the frontal region and the sinus maxillaris; including the sinus frontalis in 56 per cent of cases and some of the cellulæ ethmoidales anterior in nearly all cases.

In the cases where the infundibulum ethmoidale does not end in a pocket dorsally (fig. 30), much of the fluid that would otherwise be directed into the sinus maxillaris by this pocket, passes from the dorsal termination of the infundibulum ethmoidale into the meatus nasi medius. This, however, makes little difference—the very fact that some of the fluid gets into the sinus maxillaris makes the condition similar to the above. It requires merely more time to accomplish the same end result—a filled sinus maxillaris.

In case the ostium maxillare is not patent, the fluid after reaching the dorsal end of the infundibulum ethmoidale rises in the channel and finally passes through the hiatus semilunaris into the meatus nasi medius.

That the sinus maxillaris, because of its position and relations, is a reservoir for some or all of the fluid coming to the DORSAL END of the infundibulum ethmoidale, is a fact that admits of no debate (the ostium maxillare being patent).

IMPORTANT NERVE RELATIONS OF THE SINUS MAXILLARIS.

The roof or orbital wall of the sinus maxillaris is traversed by the infraorbital sulcus and the infraorbital canal. These passageways transmit the infraorbital vessels and nerve (considering the maxillary nerve as the infraorbital nerve from the proximal end of the infraorbital sulcus on). As a rule the canal has comparatively thick walls, but in many cases the inferior wall of the canal is of a papery delicacy and is easily compressed against the contained nerve and vessels. Frequently the canal is replaced by a groove, with the opening of the groove directed towards the sinus maxillaris. The structures—infrarobital nerve and vessels—contained in the groove are merely covered with the mucous membrane of the sinus.

The posterior superior alveolar (dental) nerves, branches of

the maxillary nerve, in most of my cases were found to pass inferiorly and ventrally upon the infratemporal surface of the maxilla, through the alveolar foramina into the alveolar canals. They thus aided in the formation of the superior dental plexus of nerves. Occasionally some of the branches of these nerves instead of taking the above course, passed entirely through the infratemporal surface of the maxilla into the sinus maxillaris. They then passed under cover of the mucous membrane of the sinus inferiorly and ventrally to the sinus floor; thence to the superior dental plexus.

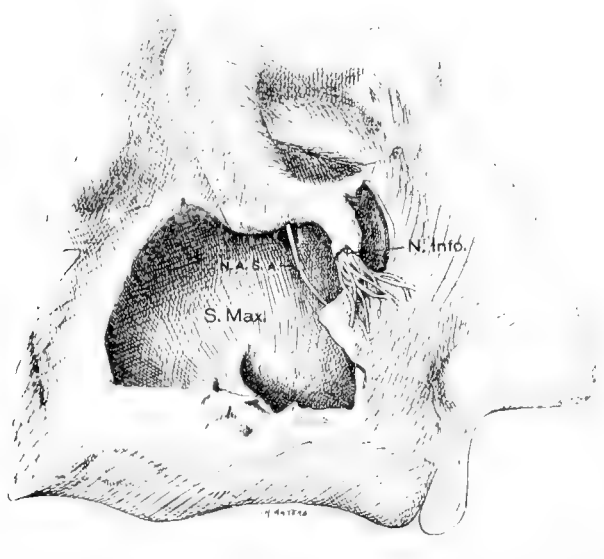


Fig. 31 (× .633). Drawing from a dissection showing the anterior superior alveolar nerve (N.A.S.A.) passing diagonally from the roof or orbital wall of the sinus to the ventral or facial wall. The nerve in this position is suspended freely in the cavity of the sinus maxillaris merely covered with mucous membrane.

N. A. S. A. = nervus alveolaris superior anterior; **N. Info.**, = nervus infraorbitalis; **S. Max.**, = sinus maxillaris.

The middle superior alveolar (dental) nerve, a branch of the infraorbital nerve, was as a rule given off in the proximal part of the infraorbital canal. It passed inferiorly and ventrally in a canal in the lateral wall of the sinus maxillaris and aided in establishing the superior dental plexus of nerves. The nerve I found in one case to arise from one of the anterior superior alveolar nerves. It also *rarely* passed under cover of the mucous membrane of the sinus to the superior dental plexus.

The anterior superior alveolar (dental) nerve was given off from the infraorbital nerve, proximal to the infraorbital foramen. It passed inferiorly in the alveolar canal of the anterior surface of the maxilla and took part in forming the superior dental plexus of nerves. From this plexus arose the superior dental nerves which supply the fangs of the teeth, the gums, and give numerous branches to the maxilla and the mucous membrane of the sinus maxillaris.

I also observed—a very important condition—that in one case the anterior superior alveolar nerve came off from the infraorbital nerve quite a distance proximal to the infraorbital foramen. The nerve then passed through the inferior wall of the infraorbital canal and took a course diagonally across the sinus from the roof to its ventral wall. The nerve thus suspended freely in the cavity of the sinus maxillaris was surrounded merely with mucous membrane (fig. 31).

CONCLUSIONS.

1. The Anlage of the sinus maxillaris appears during the third month of fetal life as a minute epithelial sac evaginating and growing at first inferiorly, later more laterally, from the dorsal end of the primitive infundibulum ethmoidale.

2. The primitive maxillary pouch may be duplicated. In some cases this may account for the duplication of the ostium maxillare of the adult sinus, i. e., the two pouches fusing distally, leaving the two points of evagination as the adult ostia. Other duplications of the ostium may develop in a way similar to that of the accessory ostium.

3. The primitive ostium maxillare varies very much in its dimensions in different embryos. This is entirely in accord with adult conditions, since the ostium of the adult sinus has a great range of dimensions.

4. Dentition seems to influence the size of the cavity but little. The age of the child and the size of the sinus apparently progress pari passu.

5. The cavity enlarges by the simultaneous growth of the

sac and the resorption of surrounding tissue. These two processes taking place pari passu with the growth of the face.

6. In a fetus at term the ventrodorsal measurement of the sinus is about 7 mm., and in a child aged 20 months it is about 20 mm. The cavity reaches its full size from the fourteenth to the eighteenth year.

7. The following may be given as average measurements of the adult sinus maxillaris, based on the measurements of 90 adult specimens:

		mm.
1	Dorsosuperior diagonal	38
2	Ventrosuperior diagonal	38.5
3	Superoinferior	33
4	Ventrodorsal	34
5	Mediolateral	23

8. The range in capacity of the sinuses studied to ascertain this fact was from 9.5 cc. to 20 cc.; with an average of 14.75 cc.

9. In the majority of cases the sinus floor is at an inferior level to the nasal floor. This distance varies from one-half to 10 mm. Sex has little influence on this relation.

10. The number of teeth that bear a direct relation to the sinus is inconstant, due to the great difference in the size of the cavity in different individuals. The three most constant teeth in direct relation are the three molars.

11. The tooth fangs may cause the formation of elevations on the sinus floor. Occasionally the fangs of some teeth are in direct communication with the mucous membrane of the cavity.

12. Frequently the walls of the sinus are uneven, due to ridges, or crescentic projections. These prominences form pockets and recesses within the cavity. Occasionally the cavity is divided by a septum into two distinctly separate compartments, each having an independent opening into the nasal fossa, but not communicating with each other.

13. The adult sinus varies much in size in different individuals, and the asymmetry on the two sides of the same individual is often marked.

14. Age, sex, and side influence the size of the adult sinus but little.

15. The adult ostium maxillare varies much in size. It is located in the superior and ventral part of the base of the cavity, and serves as a means of communication between the sinus maxillaris and the infundibulum ethmoidale. Occasionally it replaces the greater portion of the lateral wall of the infundibulum ethmoidale, and represents a slit-like aperture. The ostium may be duplicated.

16. The ostium maxillare accessorium is of very frequent occurrence. It serves as a means of direct communication between the sinus maxillaris and the meatus nasi medius. In my series of specimens it was present in 43 per cent of cases. The aperture was not found in the fetus and infant.

17. Most of the accessory ostia do not look pathological, and the writer believes that we must, in many cases, look elsewhere than to a pathological process for the determining factor in this condition.

18. Of the specimens studied to ascertain the fronto-maxillary relations, 56 per cent showed that the infundibulum ethmoidale was intimately related with the nasofrontal duct or with the sinus frontalis directly,—in case the nasofrontal duct was wanting; 40 per cent showed that the nasofrontal duct communicated directly with the meatus nasi medius—the infundibulum ethmoidale ending blindly or in an air cell; 2.5 per cent showed two nasofrontal ducts, one continuous with the infundibulum ethmoidale, and the other communicating with the meatus nasi medius; 1.25 per cent showed a direct communication between the sinus frontalis and maxillaris.

19. Since the infundibulum ethmoidale receives the ostium maxillare at its dorsal and inferior end in all cases, and the nasofrontal duct, or the sinus frontalis directly, at its ventral and superior end in over one-half the cases, it very frequently serves as a gutter-like channel, of varying depth and efficiency, communicating between the frontal region and the sinus maxlaris.

20. The sinus maxillaris, therefore, acts as a reservoir for fluids coming to the *dorsal end* of the infundibulum ethmoidale (the ostium maxillare being patent).

21. Frequently the processus uncinatus by a superior curving at its dorsal end causes the infundibulum ethmoidale to end in a pocket. This pocket is so situated that it directs fluids coming to the dorsal end of the infundibulum ethmoidale into the sinus maxillaris,—via the ostium maxillare which is in the immediate vicinity.

22. Occasionally branches of the superior alveolar nerves in passing to the superior dental plexus pass entirely through the walls of the sinus, thence under cover of the mucous membrane of the cavity to their destination. Rarely the anterior superior alveolar ramus, instead of taking its usual course, passes diagonally from the roof of the sinus to its ventral wall,—the nerve thus suspended freely in the cavity is merely covered with mucous membrane.

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THE INFLUENCE OF ALCOHOL AND OTHER ANÆSTHETICS ON EMBRYONIC DEVELOPMENT

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WITH TWENTY TEXT FIGURES

The adult nervous system is peculiarly sensitive in its responses to the influence of alcohol and other anæsthetics. The writer has found it to be equally true that alcohol and anæsthetics exert a most striking influence over the development of the central nervous system and the organs of special sense. There is considerable variation in the way in which the several anæsthetics act on the developing animal; some of them, such as ether and chloretone, producing effects of a general nature, while alcohol and magnesium are more localized or specific in their action. A similar statement is true for the actions of different anæsthetics on the adult body.

In attempting to explain the occurrence of asymmetrically monophthalmic, cyclopean and blind individuals among fish that had been developed in solutions containing magnesium, the writer advanced the hypothesis that the anæsthetic property of Mg was the causal factor. Many reasons for such a view were put forward in a paper on the artificial production of these monsters (1909). To experimentally test this hypothesis various other anæsthetic agents have been used and all of them to a higher or lower degree inhibit the development of the optic vesicles in fish embryos, and thus give rise to various ophthalmic defects. Alcohol is most decided in its action, causing in some experiments as high as 90 to 98 per cent of abnormal eyes, generally cyclopean, which far surpasses the highest results obtained with Mg.

The effect of alcohol on the general development of the nervous system is more pronounced than that of Mg, and only a few of the

alcoholic specimens ever develop sufficiently to hatch and swim about as do the Mg embryos. An explanation for this may be that Mg exerts an influence to inhibit dynamic processes, such as the out-pushing of the optic vesicles, while alcohol acts more especially on the nervous tissues. Mayer (1908) has shown that Mg inhibits muscular contractility without affecting in any way the nervous impulse or nervous rhythm.

The eye defects, it must be remembered, have only been obtained in solutions of one or another anæsthetic; the many other salt and sugar solutions which have been experimented with during four years ('06 and '07) have failed entirely to produce similar results.

The most important outcome of these experiments has been to prove conclusively that many monsters which occur in nature may be artificially produced by changing the environment of the normally developing eggs. The present experiments will demonstrate that this may be done even after development has proceeded for some time. These anomalous structures being the results of external influences and not germinal variations are to some extent within scientific control. A promising field is thus opened in the devising of means to control or regulate the development of the embryo and possibly to obviate certain monstrous conditions at least. Such possibilities were of course beyond our reach if defective germ cells were actually the cause of these monsters.

Mall ('08) has brought forward evidence to show that improper placentation or unfavorable developmental environment is responsible for most human monstrosities, many of which are aborted before reaching term. There is evidently much need of investigation aiming toward the control and regulation of the developmental environment of mammals.

METHOD AND MATERIAL

The eggs of the fish, Fundulus heteroclitus, were used in all of the experiments. The method of treatment varies somewhat for the different solutions employed, so that it is best to describe each separately.

Alcohol solutions were prepared in sea-water on the percentage basis. The strength used being 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 per cent, 60 cc. of each solution was poured into finger bowls and from 60 to 100 eggs, in the early cleavage stages, four and eight cells, were placed in each bowl. The stronger solutions killed all of the eggs, and those from 3 to 9 per cent gave the best results. In the 3 per cent alcohol solutions at times as many as 90 in every 100 embryos showed abnormal conditions of the eyes, being either eyeless, asymetrically monophthalmic or cyclopean, while in one experiment in a 5 per cent solution of alcohol in sea-water, there were 146 ophthalmic monsters against only 3 individuals with two separate eyes.

Chloretone of 0.1 per cent and 0.066 per cent in sea-water caused abnormalities similar to those produced by other anæsthetics. This substance is more general in its anæsthetic action than either alcohol or Mg, as will be seen in the discussion to follow.

Ether and chloroform also produce rather general effects on the developing embryo, yet a small percentage of cyclopean monsters occur among the embryos which are treated with 60 cc. of sea-water to which 2,2.5, and 3 cc. of ether has been added. In solutions of chloroform of about the same proportions and slightly weaker, a few monsters occurred of the type common to the other anæsthetics. Chloroform is rather toxic in its action on these eggs, large numbers dying in the weaker solutions, while others are so inhibited in their development, that various abnormal conditions follow.

Eggs were not exposed to any of the above anæsthetics for more than twenty-four or thirty-six hours. They were then placed in pure sea-water and continued development showing the abnormal conditions of the eyes and central nervous system that had been induced by their sojourn in the unusual environment.

Similar Mg solutions to those formerly employed were again used. A gram-molecular solution of $MgCl_2$ in distilled water was titrated and kept, to be diluted with sea-water to the proper strength just before the eggs were placed in it. The most

favorable results were obtained in solutions of 16, 17, 18, 19, 20, 21 and 22 cc. of molecular $MgCl_2$ made up to 60 cc. by the addition of a sufficient amount of pure sea-water; e.g., 44 cc. of sea-water was added to the 16 cc. of molecular $MgCl_2$ and 43 cc. of sea-water to the 17 cc. of $MgCl_2$. The solutions are, therefore, $\frac{16}{60}$, $\frac{17}{60}$, $\frac{18}{60}$, etc., parts $MgCl_2$ to sea-water. In the $\frac{21}{60}$ solution 66 per cent of the embryos were cyclopean in one of the experiments. Eggs were exposed to the action of Mg shortly after fertilization and at various other times until they reached an early periblast stage, or were fourteen hours old, all with similar results. Although the most favorable time for introducing the eggs into Mg solutions is during the eight-celled stage. The developing embryos were returned to pure sea-water after the third day. The Mg is so slightly toxic that eggs may be kept in it and will continue to develop; the embryos actually hatch and swim about in the solution, being, however, slightly slower in their developmental rate and not so hardy as the specimens which are returned to the sea-water.

The Action of Alcohol on Development

Weak solutions of alcohol exert a most decided effect on the developing fish embryos, causing deformities of the central nervous system, the eyes, and ears in a very large percentage of the specimens.

a. Defects of the eyes

Typical cases of cyclopia showing in the different specimens all gradations, from merely closely approximated eyes, hour-glass eyes with two pupils and two lenses, oval eyes having the two component intimately associated, typical median cyclopean eyes with scarcely an indication of their double nature and extremely small ill-formed cyclopean eyes, were present in the weak alcohol solutions. All of these have been fully described in a former paper ('09) on the artificial production of cyclopea as a result of the action of Mg. The alcohol monsters in some cases also present various degrees of the monophalmicum asymmetricum

defect which was common in the Mg experiments. Individuals may have one normal eye and the other eye in different conditions of arrested development from slightly small and defective to entirely absent, see figs. 1 and 2. An important point that was brought out by the alcohol monsters which was not noticed in the magnesium specimens is the fact that some of the embryos have both eyes equally small and defective, figs. 3, 8, 9, 10, and 11. The two eyes are symmetrically defective and the head appears to have small eye-spots instead of normal eyes; compare figs. 3, and 7, 9, 10 and 11 and 12. Finally, as in Mg solutions so also in the alcohol, many eyeless individuals are present .

The eye in some of the alcohol monsters is rather different from that found in the Mg embryos, and may possibly serve to indicate something of the condition of the eye anlagen in the brain. Many embryos possess optic cups with their concave surfaces facing almost directly toward the median sagittal plane of the head. In life the eye presents a heavily pigmented solid convex surface to the side of the head instead of the usual open pupil through which the lens may be seen within the cavity of the optic cup. Fig. 5 and 6 show front and lateral views of such an embryo; the side view indicates the peculiarly solid convex object seen when looking towards the lateral eye. Fig. 13 represents a section through this eye. The choroid coat of the eye-ball is pressed close against the body wall on the sides of the head and the concave retinal surfaces which should face outward are turned directly towards one another; a lens lies between the two eye components which are really separate except along their dorsal borders. Fig. 4 shows a somewhat similar specimen in which the eyes are entirely separate yet they have an arrangement almost identical to that just described. The eyes face the mid-plane of the head and turn their convex choroid coats out against the body wall. The only place at which the inner face of the eyes touches the ectoderm is the ventral body wall and from this a lens has arisen and lies between the two eyes. Fig. 14 illustrates a section through these eyes, in which a most peculiar arrangement exists. Optic fibers probably arising from the ganglionic layer of the retina, (although this can not be positively demonstrated in the sections),

Camera Drawings of Living Fundulus Embryos

Fig. 1. An embryo twenty days old which was treated with 5 per cent alcohol. Only one eye is formed and it faces in a ventro-median direction, instead of towards the lateral wall of the head.

Fig. 2. An asymmetricum nonophthalmicum monster with one almost typical eye while the other eye is small and poorly formed, from a 4 per cent solution of alcohol in sea-water.

Fig. 3. An embryo with both eyes small and closely approximated; the eyes face ventrally. This type is common in all of the anæsthetic solutions.

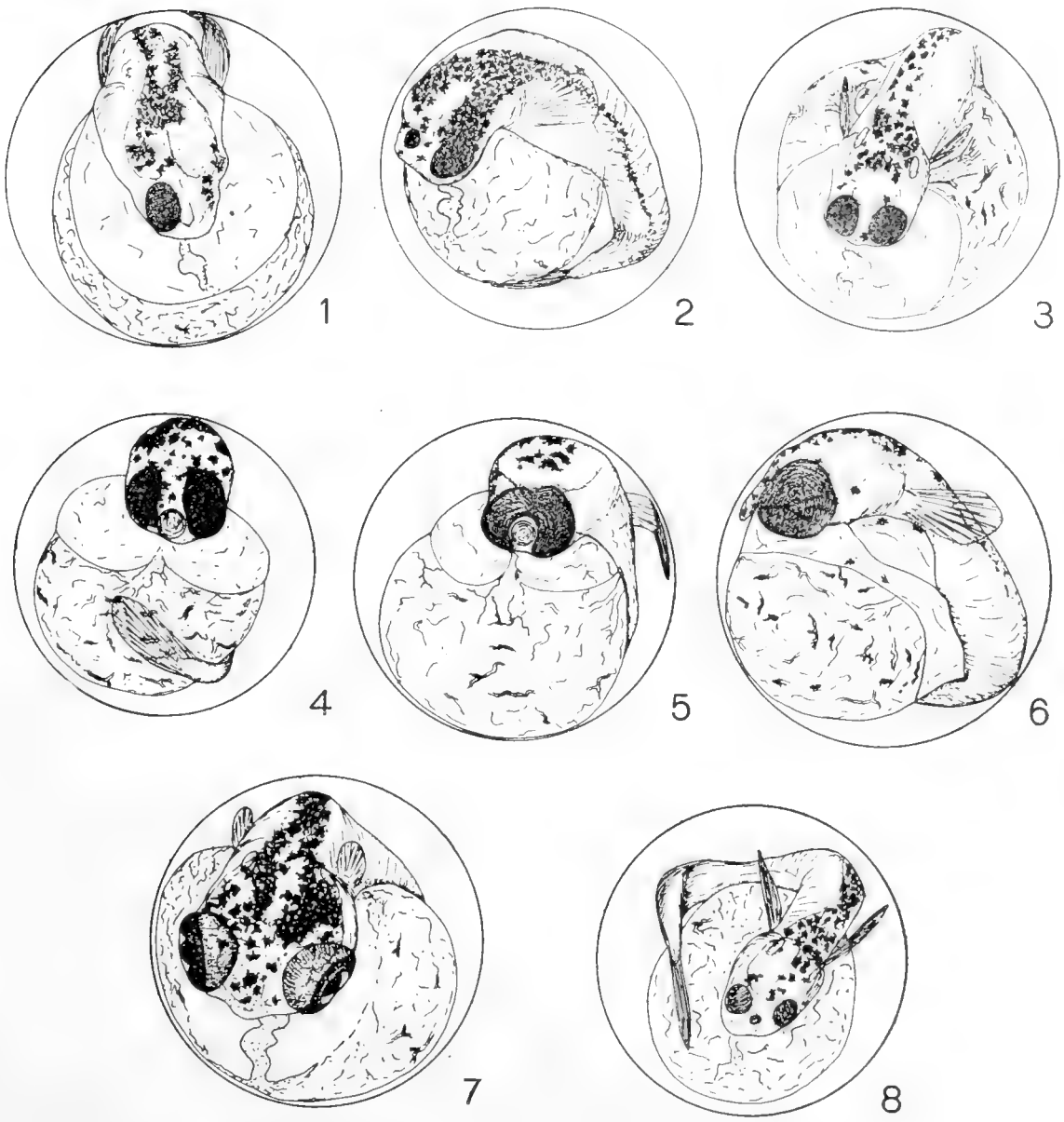
Fig. 4. A nineteen day embryo from 5 per cent alcohol. The two eyes are not connected yet their convex surfaces are turned out against the lateral walls of the head and the pupils face the median plane. A single lens lies between the two eyes. Fig. 14 shows a section of these eyes.

Fig. 5. A nineteen day embryo from 5 per cent alcohol. This front view shows the two eyes joined dorsally and facing one another with the lens between them.

Fig. 6. shows a lateral view of the same head with the convex choroid surface of the eye-ball close against the side of the head. Fig. 13 illustrates a section through these eyes.

Fig. 7. A normal Fundulus embryo when eight days old drawn to the same scale as the monsters.

Fig. 8. A twenty day embryo from 5 per cent alcohol; the eyes are small and defective as shown in the section fig. 10.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are collected into optic nerves which pursue extraordinary courses; instead of passing through the outer retinal layers and the choroid coat they take an almost directly opposite course and run across what should be the optic cup cavity (the humor cavity in these specimens is filled with loose cellular tissue) and out through the wide-open "pupil," forming a perfect cross and then passing into the base of the brain to end in the optic lobes.

The position of the lens must not be supposed to determine the actual pupil region of these eyes, as the lens clearly lies between them; see fig. 4 of the living embryo. The wide open pupils of the two eyes face or lead directly into one another. The position might be taken that the entire arrangement represents one large eye; this is not true however since the eyes are entirely separate in all of the sections and the optic cross could scarcely be expected to exist within the base of the eye itself as would be the case if this were one huge eye. The eyes really hang down from the brain as two large retinal disks. Fig. 11 represents a similar case with the eyes rather more flattened out laterally, and the existence of all gradations between the two conditions substantiates the above statement.

The retinal layers of these eyes which are nineteen days old, are poorly differentiated, the inner layer consisting merely of indefinitely arranged cells; a better differentiation is usually attained in normal specimens by the sixth or seventh day. A comparison of Figs. 13 and 14 with Fig. 12 illustrates in a way the more definite orientation of the inner retinal cells in the normal individual when compared with the defective eyes. It will also be noticed that the lenses in the two-eyed specimen are surrounded by clear humor spaces while the lens in the defective eyes lies buried in loosely arranged cellular tissue.

The right retina of the monster faces in the same direction as the left retina of the ordinary individual yet there is no indication of a reversal of the layers which might possibly be imagined in such a case.

The optic stalk was scarcely formed in these eyes, which is not infrequently true in cyclopia. The path of the optic nerve is, therefore, evidently not that usually taken along the

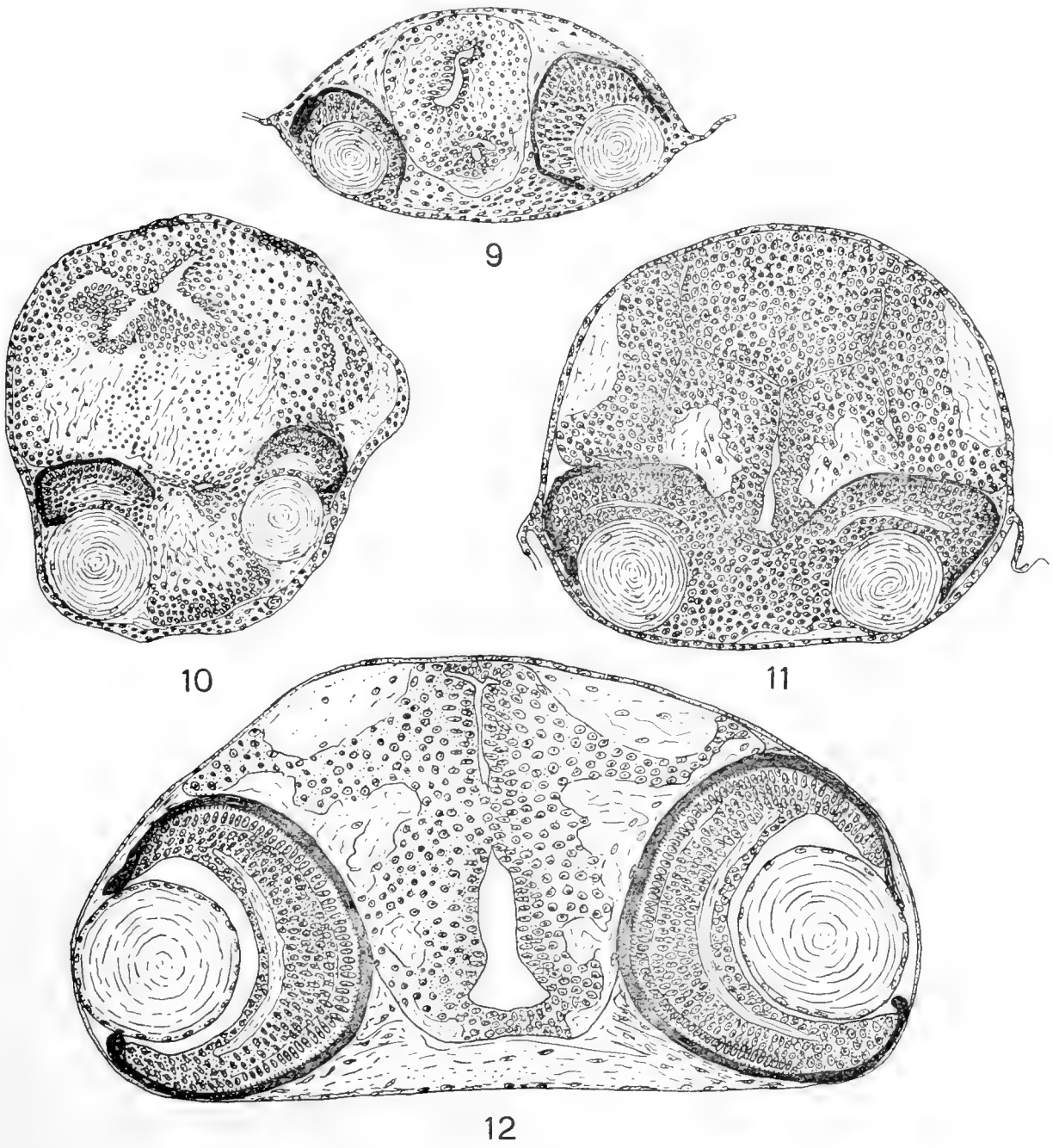


Fig. 9. A section through the small defective eyes of an eight day embryo, after treatment with a 4 per cent solution of ether in sea-water. The brain is narrow and poorly developed.

Fig. 10. A section through the head of the embryo shown in fig. 8. Both of the eyes are small and defective with ill-fitting lenses and face in a ventral direction.

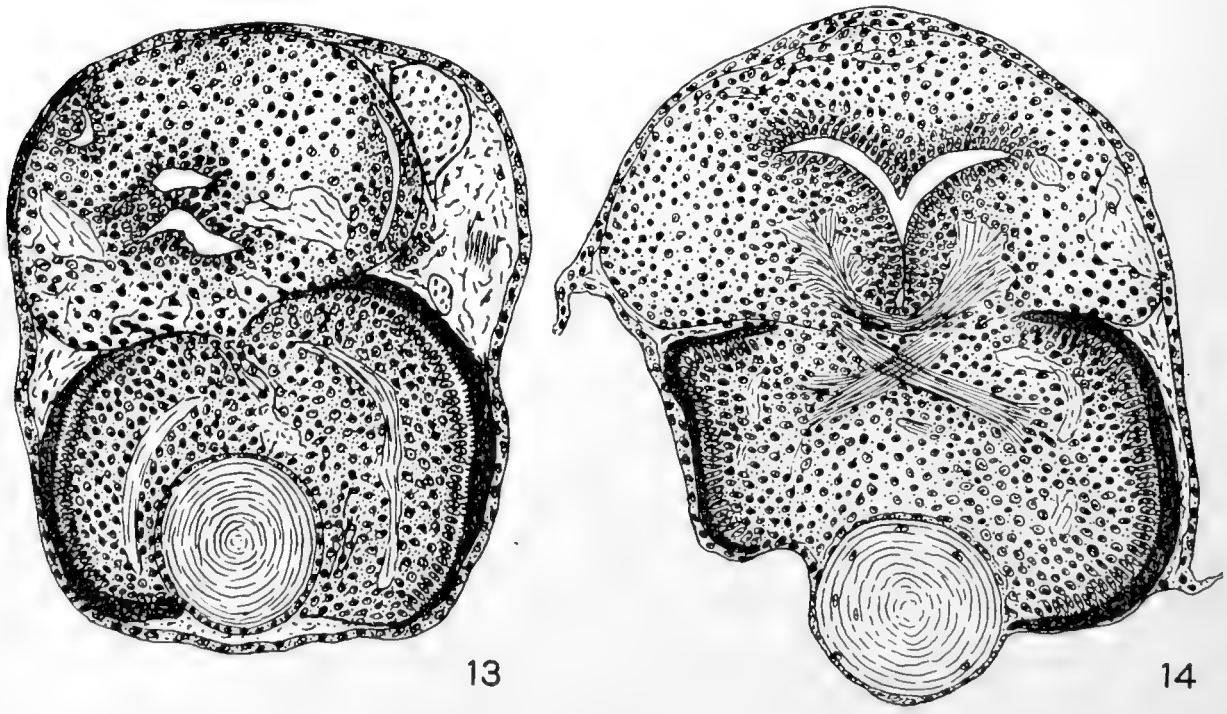
Fig. 11. A section through the eyes of a monster commonly found in the alcohol solutions. The eyes are joined beneath the bilateral brain and face ventrally.

Fig. 12. A section through the eyes of a normal thirteen day embryo.

optic stalk, but the optic nerve fibers grow directly into the brain from the region of the eye cavity itself.

It is interesting to find in this connection that Lewis ('70) describes in his experiments on tadpoles a strikingly similar course pursued by the nerves arising from some of the optic cups he had transplanted to various positions along the hind brain region of the embryos. He states that

In a few of these somewhat irregular transplanted eyes the optic nerve takes a very curious course, passing across the cup cavity from the ganglionic layer, through the pupil and then into the mesenchyme, ending there. In both of these experiments a small bundle of optic nerve fibers pierces the retina as far as the pigment layer. In transplanting these eyes the ganglionic layer was probably injured in such a way as to interfere with the normal path of the nerve fibers, and so they have probably followed the path of least resistance through the pupil and out into the mesenchyme.



13 14

Fig. 13. A section of the embryo shown in figs. 5 and 6. The optic cups are joined dorsally and face the median plane of the head; a lens lies between them and is surrounded by loose cellular tissue instead of by the humor.

Fig. 14. Section of the eyes in the embryo shown in fig. 4. The optic cups are not joined yet they face towards one another with their convex choroid surfaces pressed close against the head wall. The optic nerves run across what should be the humor chamber of the eyes and out through the wide pupils to form a perfect cross; the optic fibers then enter the brain floor. A lens lies between the eyes.

Lewis' illustrations are similar to Fig. 14 in so far as the course of the optic nerve is concerned. These experiments would seem to indicate that the direction taken by the optic nerve fibers is not firmly fixed but that they may pursue an almost reverse direction from that generally followed. Many of the eyes in the writer's specimens show various conditions of this kind and he must agree with Lewis in the conclusion that

> It would seem to me impossible to explain these various conditions of the optic nerve on any other basis than that they are outgrowths of nerve cells of the ganglionic layer of the retina.

Direct evidence is thus furnished for the outgrowth theory of the nerve fiber which has been so ably supported in the last few years by Harrison's ('08) experiments.

The present experiments warrant the following explanation for incomplete cyclopean eyes, or double eyes, when compared with the usual condition.

In normal development the eye anlagen push out from the ventro-lateral borders of the brain and turn dorsally as indicated in the diagram, fig. 15 A. The abnormal individuals with two eyes facing the median plane also have them more ventrally situated in relation to the brain, and it may be supposed that when the eyes arose from the brain their formation was directed ventrally instead of dorsally, fig. 15 B. This causes the eyes to hang below the brain and face one another as already shown in figs. 4, 5, 6, 13, and 14, instead of turning dorsally and facing outward as in figs, 11 and 12.

Similar conditions are also found in the development of a single eye. Fig. 1 shows an embryo with an eye on the right side only, yet this eye faces the median plane and is unusually ventral in position; it probably arose as indicated in the diagram fig. 15 D, where as other single-eyed individuals, the commoner type, have an eye looking out from the usual lateral position, fig. 15 C.

From these conditions we may determine whether cyclopia is brought about by a failure of certain central tissues of the brain to develop, thus allowing the eye anlagen to come together as Lewis ('09) has suggested, or whether through a lack of developmental energy necessary for the optic cups to grow dorsally and

outward to meet the ectoderm as the writer ('09) has supposed. Considering the case of the single eye it might be held that the failure of certain central tissues of the brain to develop would cause the eye to arise too near the median line, but this lack of central tissue does not explain why the eye faces the median plane instead of the lateral head wall, and it is much less able to account for the absence of the eye on the opposite side of the head. On the other hand, if the conditions are due to a lack of the necessary developmental energy or an anæsthesia produced by the experiment, then it is evident that although one eye does succeed in pushing out from the brain it might not have sufficient developmental energy to grow dorsally and outward to the lateral body wall, but droops, as it were, into a more ventro-median position and faces in toward the median plane. Thus one-half of an incomplete cyclopean eye is formed. The other eye was entirely suppressed, lacking the energy necessary to push itself out from the brain. This inequality in the developmental powers of the two eyes is indicated by their frequent asymmetrical condition.

The two eye components do not always face the median plane and in such cases the eyes merely fail to grow out laterally. They come off ventrally from the brain and either face in a ventral direction or grow so as to face outward.

The experiments fail to give any definite clue as to where the optic anlagen are located in the brain before they become visible, although Lewis' operations on the embryonic shields of older embryos would seem to indicate that at that time the anlagen occupied somewhat lateral positions.

It is clear from the foregoing consideration that alcohol has the power to induce the same typical ophthalmic defects that were formerly described in the embryos from the Mg solutions. The property common to both Mg and alcohol is their anæsthetic effect on animals. The writer concludes that cyclopia, monophthalmicum asymmetricum and entire absence of eyes, all of which are more or less arrested or inhibited condition of development, result from anæsthesia during certain embryonic stages. Of course this may not be the sole cause of such defects; on the contrary the fact that they are produced in this way would indicate

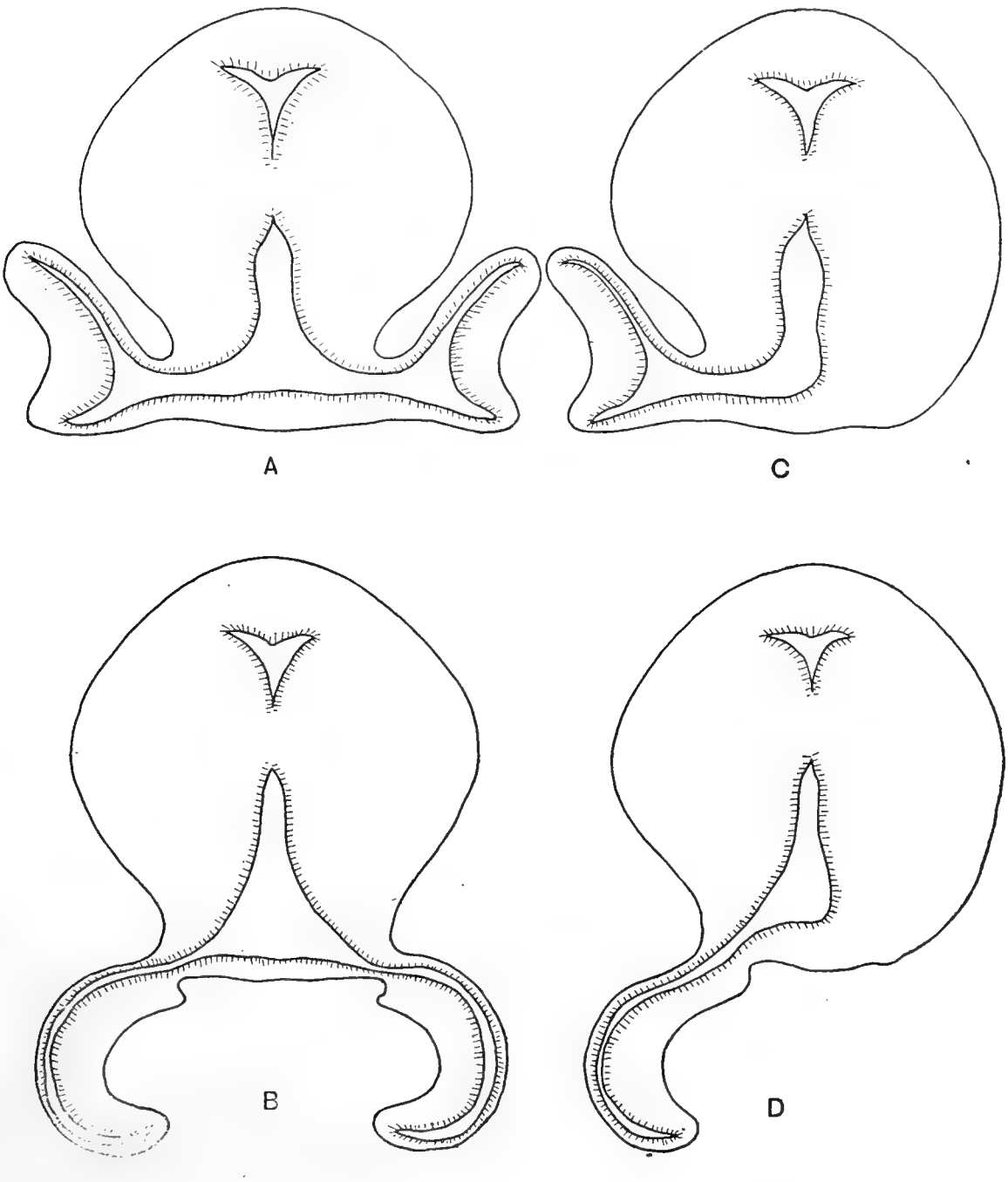


Fig. 15. A diagram illustrating several positions taken by the optic cups in development. A, the usual case in which both cups push out from the brain and turn dorsally so as to face the lateral head wall with their convex choroid surfaces towards the brain; see fig. 12. *B*, the optic cups push out from the brain but instead of growing dorsally, they hang ventrally and face in towards the median plane, with their convex choroid surfaces against the outer head wall; see figs. 13 and 14. *C* and *D*, the same conditions are often realized when only one optic vesicle arises from the brain.

that any factor which might come in during early development to lower the developmental energy could possibly induce similar defects. In mammals such monsters probably arise as the result of some weakening or debilitating influence of the environment during early developmental stages, which need only have acted for a short space of time.

b. Defects of the auditory organs

A very pronounced suppression in the development of the auditory apparatus is often noticed in the embryos which have been treated with weak solutions of alcohol. In many individuals only one ear exists. When this condition is found in an embryo with only one eye, two unequally developed eyes or a cyclopean eye with asymmetrical components, it is of interest to find that in all cases observed, the ear is on the same side of the head as the better formed eye. In rare cases both ears are absent, and again it often happens that the ears are apparently normal while the eyes are deformed. Fig. 16, a horizontal section through the head, shows two small abnormal eyes with a lens between them and two perfect ears with cartilaginous capsules, near the hind brain. Fig. 18, which is a section through the ear region of the embryo shown in fig. 4, illustrates two poorly formed ears; on the right side the ear is small and two semicircular canals are represented only by their ampullæ, the epithelial lining of which forms papillæ of cells with long hair-like processes growing from them as is indicated in the drawing. The left ear is almost entirely absent, its median section showing only the small cavity and ampullary papilla seen in the figure. Both ears, however, are surrounded by well formed cartilaginous capsules.

A remarkably abnormal ear is seen in fig. 17. The auditory vesicles have united so as to occupy a dorsal position above the posterior end of the brain. Only two semicircular canals are developed on each side. The cartilaginous capsules in this case seem unable to meet the situation and extend for only a portion of the way around the huge auditory cavity. This union of the lateral auditory vesicles, although formed by an entirely different principle, suggests the large double cyclopean eye.

The final persistence of the ampulla-like cavities seems to be the rule, these structures being present even when all other portions of the internal ear are absent. The ampullæ of the canals are perhaps particularly useful as organs of equilibration in these animals, and their stuborn persistence may be indicative of an ancient origin and suggests the primary function of the ear as an organ of equilibrium.

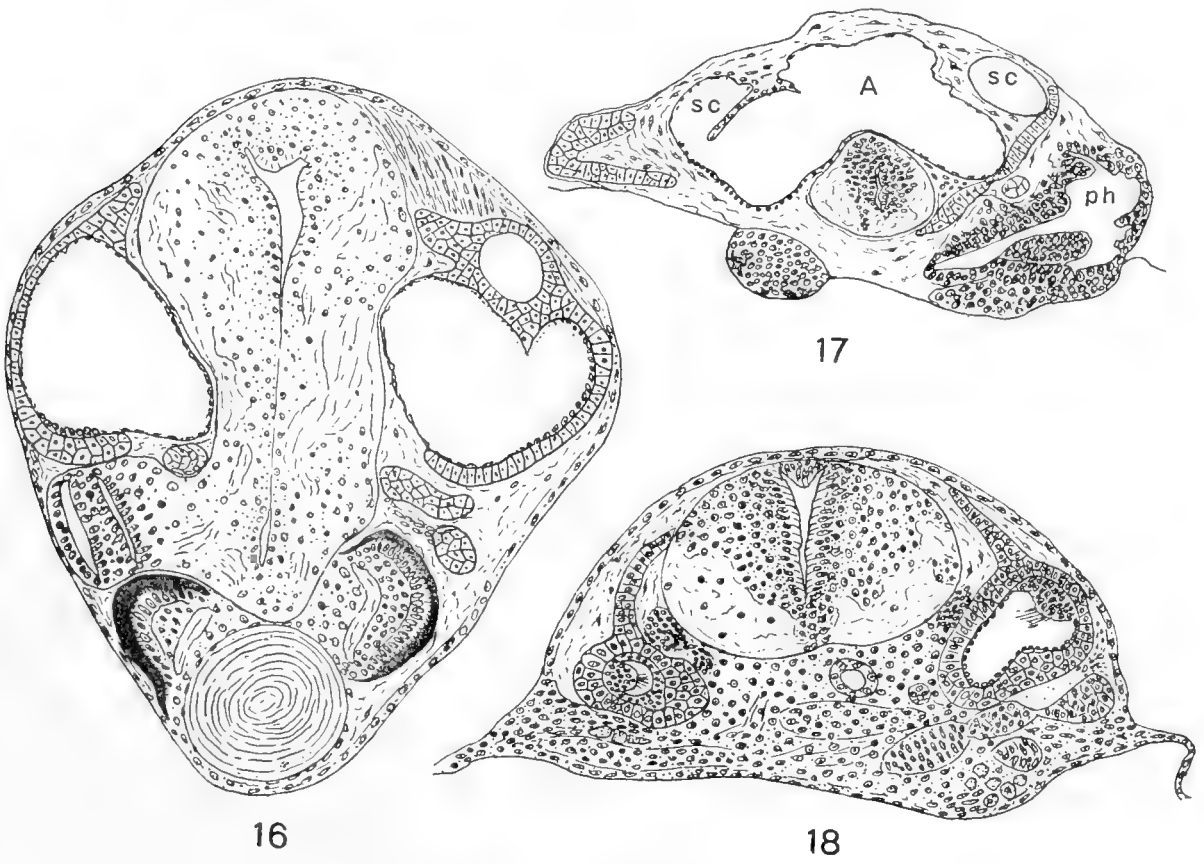


Fig. 16. A horizontal section through the head of a thirteen day embryo from a 5 per cent alcohol solution. Two very small and defective eyes have alens between them, while more posteriorly two perfectly formed auditory vesicles are seen with cartilaginous capsules surrounding them.

Fig. 17. A section through the auditory region of an eight day embryo treated with a 4 per cent ether solution. The two auditory vesicles unite dorsally to form a huge cavity above the medulla and spinal cord. This embryo also shows an incomplete cyclopean eye and spina-befida. sc, semicircular canal; a, auditory vesicle,; ph, pharynx.

Fig. 18. A section through the auditory vesicle of the embryo shown in fig. 4 and section fig. 14. The entire auditory vesicle is suppressed except the ampullæ of the semicircular canals. The right ampulla is larger and shows a papilla with hair-like fibers, while the left ampulla is almost completely closed, yet, it too, shows the papilla with projecting hairs. The cartilaginous capsules are small and thus adjusted to the tiny ear parts.

Although all parts of the ear are absent the cartilaginous capsules are present. The shape and size of the capsules, however, seem to be adjusted to that of the auditory vesicle when any part of it exists, as is indicated on the two sides of fig. 18.

c. Defects of the central nervous system

The abnormalities of the brain shown by the specimens treated with alcohol might easily form the subject of an extensive monograph so various and numerous are they. Only a few of them will be briefly mentioned.

In rare cases the brain is almost normal; the fore brain, however, is usually very narrow and gives to the head a characteristically pointed appearance. Dorsal herniæ at times occur in the region of the optic lobes and the hind brain. The histological structure of the brain is often peculiarly abnormal in both the arrangement and the appearance of the cells. The cells may be hyaline and in the region of the central cavity fail to take the stains. They may even be diffusely scattered in peculiarly defective specimens.

The spinal cord in some individuals also shows the hyaline appearance about its central canal, and spina-bifida in not uncommon. The latter condition no doubt results from the general inhibition in rate of development which is constantly true for the specimens in the alcohol solutions. The germ ring is slow in surrounding the yolk and consequently the trunk region of the early embryo is abbreviated. This condition interferes with the median cell proliferation forming the spinal cord so that a split or divided cord results and may extend for various distances in the trunk region. Fig. 19 shows a section through a trunk region with a double cord, *ch*, the notocord, *nch*, is also divided. Fig. 20 is a more posterior section of the same embryo and shows the cord and notocord again single as they are in more anterior region than that shown by fig. 19.

Many of the defects of the central nervous system are of a general nature and almost any substance that inhibits or interferes with the normal developmental rate may cause them. The writer does not intend to convey the idea that these are characteris-

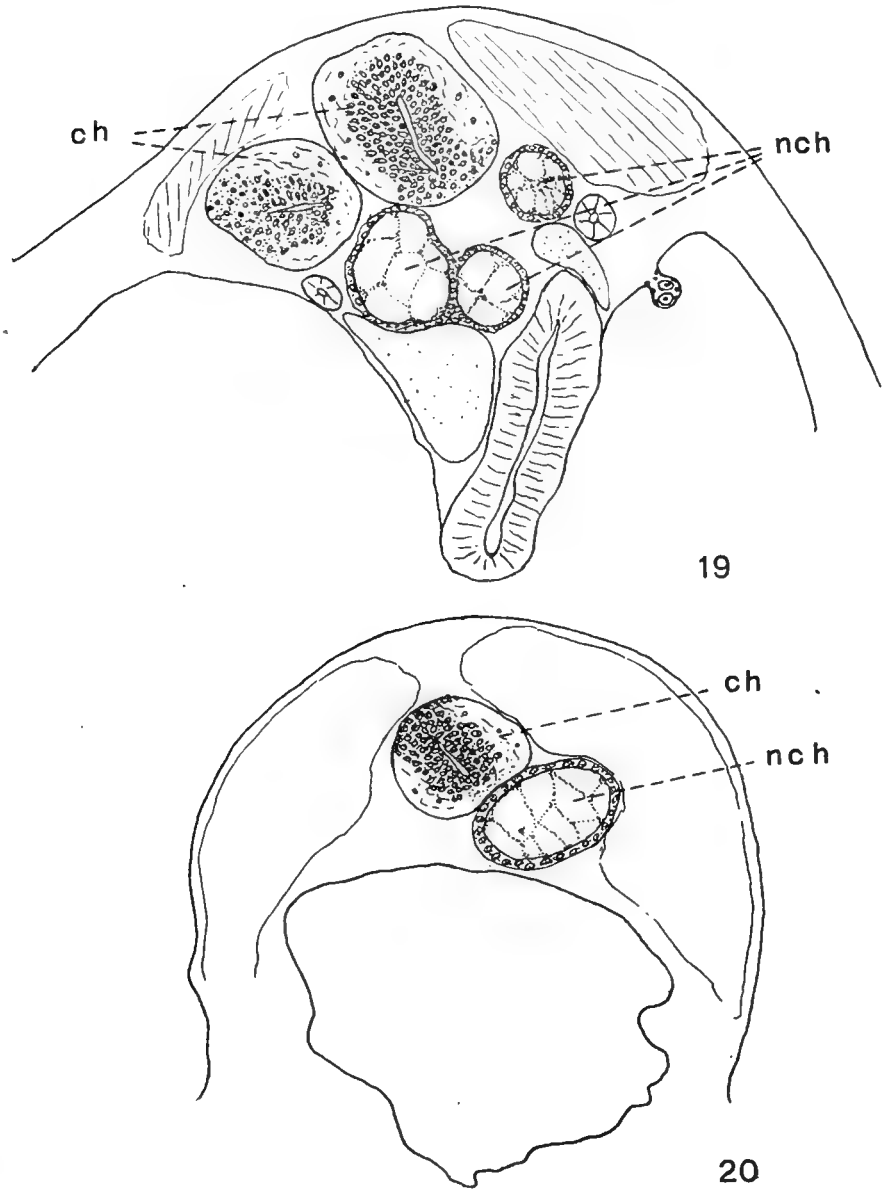


FIG. 19. A section through the trunk region of an eight day embryo from a 4 per cent ether solution. The spinal cord, **ch**, is double (spina-bifida) and the notochord, **nch**, is divided into three parts.

FIG. 20. A more posterior section of the same embryo, showing the spinal cord and notocord again single as they are in regions more anterior than fig. 19.

tic anæsthetic effects, but they are strikingly common in the alcohol solutions. On the other hand, similar abnormalities of the nervous system are really infrequent in the weaker Mg solutions.

The slight effects of Mg on the development of the central nervous system are interesting when compared with the marked effects of other anæsthetics on these tissues. In this regard it is important to remember that in physiological experiments on nerve-muscle preparations Mayer ('08) has found that Mg acts directly as an inhibitor of muscular activity, exerting little if any effect on the activity of the nervous parts. The action of Mg in these experiments is not particularly on the nervous system but more largely on the dynamic processes concerned in the outpushing of the eyes.

The occurrence of the several ophthalmic anomalies is common to all of the anæsthetic solutions and similar conditions have not been found in embryos treated with various salts and sugars ('06, '07) which may inhibit general development and induce many other abnormalities.

The Effects of Chloretone, Ether and Chloroform on Development

Chloretone, ether and chloroform when employed in weak solutions influence developing eggs in a way somewhat similar to that described for alcohol. The action of these substances is not so pronounced and may be described more as an inhibition of the general developmental processes. The embryos are usually small and recover very slowly from the inhibiting effects after being returned to sea-water. A few of the individuals in all these solutions exhibit the cyclopean defect in its various degrees just as it has been described in the specimens treated with alcohol and Mg.

The ophthalmic defects, cyclopia, monophthalmicum asymmetricum and entire absence of optic vesicles, are all conditions of arrested or inhibited development and are prevalent among embryos treated with solutions having anæsthetic properties.

The writer is led to conclude, therefore, that his former hypothetical explanation of why cyclopia occurred in embryos treated with Mg solutions was correct. The evidence strongly indicates that the ophthalmic abnormalities produced in these experiments are the result of an anæsthetic action during the early developmental stages.

THE PERIOD OF DEVELOPMENT AT WHICH CYCLOPIA MAY BE INDUCED BY CHEMICAL AGENTS

The Mg experiments were repeated mainly to ascertain at how late a period in development eggs could be subjected to the solutions and subsequently develop into cyclopean monsters. The original experiments seemed to demonstrate the fact that cyclopia was due to external influences acting on the development of the optic vesicles, and not in any sense to a germinal variation. Nevertheless, H. H. Wilder ('08) in the face of these results, advanced a germinal theory to account for the origin of cyclopia and attempted to explain away the obstacle offered in the experiments referred to by claiming that the eggs were subjected to the solutions at so early a stage in development that germinal variations might still have been induced. This is impossible, as the writer has pointed out elsewhere ('09b), since germinal variations may only be induced before embryonic development has begun. After the two-or four-cell stage (the time at which the eggs were subjected to Mg) is reached any thing done to the egg has its effect on the developing embryo to cause this or that abnormal condition. The only germinal variation possible at such a period would be in the primordial germ cells of the developing individual, a variation which would not manifest itself until the next generation of individuals.

The following experiments prove beyond doubt that cyclopia may be produced by the action of environmental influences.

When eggs in the eight-cell stage, or three and one-half hours after fertilization, are subjected to the action of Mg solutions many of the resulting embryos will show the cyclopean defect.

If eggs be placed in Mg solutions five hours after fertilization, when in the sixteen or thirty-two-cell stage, an almost equally large number of cyclopean individuals will occur. The same is true when they are subjected to the solutions after having developed in pure sea-water for seven and one quarter hours and reached the sixty-four or higher cell stages.

Eggs that have developed eleven hours in pure sea-water and are in the early periblast stage with a somewhat flattened blastodermic cap, may be put into Mg solutions and caused to form cyclopean monsters. The percentage of cyclopean embryos arising from eggs treated at this late period is small, yet even after developing for fifteen hours in pure sea-water some eggs may be induced to form cyclopean monsters by treatment with $MgCl_2$ in sea-water solutions.

Eggs that were older than this before being introduced into the solutions failed to respond, all developing into ordinary two-eyed individuals. This is due to the fact that a considerable amount of time is necessary for the Mg to act upon the body substances of the early embryo and prevent normal eye development. The optic vesicles begin to push out from the brain before the thirtieth hour in development. Thus, after the first six or seven hours, the longer the eggs have been allowed to develop naturally the smaller the proportion of cyclopean individuals that may be artificially induced. After fifteen hours no embryo will be so affected, since an insufficient amount of time exists for the Mg to act on the eye anlagen.

The solutions are effective up to a stage in development preceding the formation of the germ ring and embryonic shield, and the action of the Mg on the eye anlagen probably takes place while the embryonic shield and outline of the embryo are forming.

There can be no further doubt that cyclopean monsters are caused by the action of a strange environment on the developing fish embryos. With such evidence at hand it is also highly probable that mammalian cyclops are due to the action of external influences on the embryo and not to an abnormal germinal tendency.

OTHER CASES OF CYCLOPIA IN FISH

The Italian observer, Paolucci ('74), has described a most remarkable cyclopean ray. The monster was almost adult in size, probably two years old, and measured 47cm. across the pectorial fins and 20cm. in length not including the long whip-like tail. Paolucci states that this cyclopean monster was captured in the Adriatic Sea near the shore and had evidently been able to cope with its surroundings and grow into a vigorous ray. So far as the writer knows this case of a cyclopean monster in nature being able to sustain itself and reach the adult stage, is unique.

Paolucci's specimen proves the correctness of the writer's statement ('09) that the cyclopean eye is not necessarily associated with a single instead of a double brain, or with any other serious defect in the brain region. This fact was clearly shown in the brain structure of many of the cyclopean embryos studied, as well as by their apparently normal behavior after hatching from the egg.

The cyclopean Funduli have been kept living for more than one month, which is as long as the experiment was tried. They would doubtless have lived much longer, as they were hardy and able to obtain an abundance of food from the vegetable particles in the sea-water. Paolucci's observation would indicate that the Fundulus monsters might be reared to maturity and possibly interbreed.

Gemmel ('06) has described four cases of cyclopia in newly hatched trout collected from a fish-hatchery in England. The conditions of the eyes and brains in these monsters are exactly similar to those in the artificially produced Fundulus monsters.

The developing trout's egg demands water of such high purity that trouble is often experienced in the hatcheries, and monstrous embryos commonly occur. These may result from weakened developmental forces due to an insufficient oxygen supply or to the accumulation of injurious chemicals about the eggs.

Gemmel ('06b) in describing cases of supernumerary eyes in the trout embryos records that in one case of an aborted twin head the lens alone of all the eye structures was present. Free lenses

were also described and figured in other individuals. Free lenses occur very commonly in heads showing various eye abnormalites; a full consideration of these cases is recorded elsewhere.

SUMMARY

1. When the eggs of the fish, Fundulus heteroclitus, are subjected during early stages of development to the action of weak solutions of alcohol the resulting embryos show marked abnormalities in the structure of their central nervous system and organs of special sense.

The eyes in such individuals are either both small with poorly differentiated retinæ, cyclopean, asymmetrically monophthalmic or entirely absent. These ophthalmic defects sometimes occur in as many as 98 per cent of the specimens. Such anomalies are closely similar to those previously induced with Mg, and in both cases are probably due to the anæsthetic property of the substances acting upon the eggs.

Alcohol tends to suppress the development and differentiation of the auditory vesicles. A few specimens are entirely without ears, others have one ear more or less perfectly developed while the opposite ear is scarcely formed at all and still other individuals have both ears extremely defective. In all cases examined the better ear is invariably on the side with the better developed eye if the eyes are also asymmetrically formed. The most persistent portion of the internal ear, or that part which exists when all other parts are wanting, is a cavity with an epithelial lining resembling closely in structure an ampulla of the semicircular canals. This fact may be interpreted to mean that the ampulla is one of the most ancient or fundamental parts of the ear, and it might further be considered indicative of the archaic function of the ear as an organ of equilibrium since this is the chief function of the ampullæ.

The brain is usually narrow and pointed in embryos that have been treated with alcohol. It occasionally has a dorsal hernia and shows regions of poor differentiation. The cell arrangement in the spinal cord are abnormal in many cases and spina-bifida

is not infrequent. These conditions of the central nervous system might result from any cause that tends to retard development and are not particularly characteristic of anæsthetic solutions as the eye anomalies are; yet the defects of the central nervous system are commoner in these anæsthetics than in any other solutions with which the embryos have been treated.

2. Chloretone, chloroform and ether induce much the same structural deformities in these embryos as does alcohol. They act, however, as more general anæsthetics, causing a retardation in development. The characteristic eye and ear defects are not nearly so common, though they do occur as a result of treatment with these three substances.

3. The effects of Mg on the developing fish's egg have been previously considered. This substance is even more local in its action than alcohol, the principal defects resulting from its use being various anomalous conditions of the eyes, whereas the nervous system generally may be in many cases structurally normal. The embryos on hatching from the egg are able to swim in the usual manner and live for more than one month in aquaria, which is as long as any effort was made to keep them. The latter fact would seem to indicate that the nervous system also functionates normally.

Magnesium was used to test at how late a period in development the eggs might be introduced into the solutions with the subsequent development of the cyclopean condition. It was found that after normal development had proceeded for two, four, six, eight, ten, eleven, twelve or even fifteen hours, if the eggs were then placed in $MgCl_2$, solutions, many of the resulting embryos showed the cyclopean defect. At fifteen hours the eggs have reached the periblast stage and the blastodem is flattening down upon the yolk. The germ ring arises shortly after this time and begins its downward growth over the yolk mass.

Whenever eggs are allowed to develop beyond the fifteen hour period before being introduced into the solutions of $MgCl_2$ they invariably give rise to normal two-eyed individuals. The occurrence of cyclopia is less frequent when eggs are subjected at later stages than when introduced into the $MgCl_2$ solutions during the four or eight-cell stage. This is doubtless due to the fact that a

considerable period of time is necessary for the Mg to act upon the substances of the embryo and influence the origin of the optic vesicles. When an insufficient time intervenes between the period at which the eggs are subjected to the action of the solution and that at which the optic vesicles are given off from the brain the Mg is unable to influence the tissues so as to induce the cyclopean condition.

The production of cyclopia by the action of Mg at such late stages in development proves beyond doubt that this deformity is due to the action of external or environmental conditions on the developing animal. Any explanation of cyclopia based on germinal hypothyses such as that recently advanced by H. H. Wilder must be reconstructed so as to conform to these facts.

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THE INDEPENDENT ORIGIN AND DEVELOPMENT OF THE CRYSTALLINE LENS

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WITH TWENTY-EIGHT TEXT FIGURES AND TWO PLATES

INTRODUCTION

The crystalline lens normally arises in the embryo from ectoderm overlying the optic vesicle and continues its development in close association with the optic cup. This fact suggests that some correlation exists between the manner of development of the optic cup and the optic lens. In case such a correlation does exist, to what extent is the optic vesicle and cup responsible for the origin and subsequent development of the lens; and, on the other hand, what influence, if any, does the presence of the lens

exert over the development of the optic vesicle into the cup and its subsequent development into the eye?

If it is proven that the optic vesicle and cup possess the power to derive a lens from the ectoderm the further question then arises is the ectoderm under any condition able to give rise to a lens without the optic vesicle stimulus? If so, is this independent lens-bud capable of self-differentiation to such an extent as to form a perfectly constructed lens?

Many questions of detail, as, for example, the relationship between the size of the optic cup amd the size of the lens, the ability of partial or defective optic cups to stimulate lens formation, whether the lens may be derived only from certain areas of ectoderm or from any ectoderm, and other problems which we will presently consider, also present themselves.

The study of these questions has come to be known as the lens problem. Experimenters have attacked the questions from several sides during the last ten years and the lens problem has in many ways been beautifully analysed, yet today much additional evidence is necessary to entirely clear the situation.

In a previous paper the writer ('09) briefly described the independent origin and self-differentiation of the optic lens in the fish embryo. Since that time further experiments have yielded much additional material and evidence bearing on this subject. The results of these experiments are considered in the present contribution and show in a most convincing manner that the crystalline lens is capable of originating independently from the ectoderm and of subsequent self-differentiation. Definite proof will also show that although the lens may arise independently, nevertheless the optic vesicle invariably has the power to stimulate the formation of a lens from any overlying ectoderm with which it may come in contact.

The action of the optic vesicle on the ectoderm is a much stronger force for the production of a lens than is the innate tendency of the ectoderm to produce an independent lens. Slightly injured ectoderm may be rendered unable to form a free lens while the same weakened ectoderm will respond to a contact

stimulus of the optic vesicle by forming a lens. This point is important, for herein the writer believes, after a study of his own experiments and the results of other workers, lies the explanation of many discrepancies in the operation experiments on the lens. When the operation is so performed that the ectoderm must be folded away in order to extirpate the optic vesicle and is then returned to its place, free lenses have failed to occur, although an optic vesicle may still have been able to derive a lens from this replaced ectoderm. On the other hand, when the early open medullary plate is operated on so as to remove the optic vesicle areas, the ectoderm of the head wall is sometimes left uninjured and from it may arise free lenses. The free lenses of King's experiments arose in embryos which were operated on dorsally to burn out the optic vesicle areas of the partially open medullary tube. The lateral head ectoderm was probably uninjured in some of the specimens. In Spemann's more recent experiments the early open medullary plate was operated upon directly to remove the optic vesicle areas; in such experiments free lenses arose from the uninjured ectoderm. Experiments on other species at such stages and in a similar manner will probably give like results.

In the experiment of removing the optic cup and leaving a partially differentiatied lens, this lens may have degenerated or ceased to differentiate on account of the injury it suffered by the operation, the absence of the optic cup not affecting it. It is unquestionably true that in some amphibians and fishes the lens is capable of perfect self-differentiation.

The optic cup does not exert complete control over either the size or shape of the optic lens. Numerous points of detail are also elucidated by the study of the optic organs in artificially produced fish monsters which are either blind or present various eye defects.

The experimental part of this investigation was conducted during the summer of 1909 in the Marine Bioligical Laboratory at Woods Hole, Mass., while occupying one of the rooms of the Wistar Institute.

2 METHOD AND MATERIAL

In all former experiments on the developing lens, except those which the writer recorded ('09), mechanical methods have been resorted to in destroying the early optic vesicle. This has been accomplished by burning the region with hot needles or by cutting away the tissue. It matters not how cleverly such experiments may be performed they are often open to objection, particularly when the experimenter has to remove or injure the overlying ectoderm in order to reach and extirpate the optic vesicle.

The present experiments have been conducted in an entirely different manner. When developing fish embryos, Fundulus heteroclitus, are treated with certain magnesium salts, alcohol, chloretone or other anæsthetic agents, the development of the optic vesicles is prevented entirely in some cases, while in other specimens only one vesicle forms on either the right or left side, and finally a large majority of the embryos present the cyclopean defect with a more or less double ventro-median eye. We have here, therefore, an exceptional opportunity to study the relationship between the development of an optic vesicle and a lens. In the first case, does a lens or do lenses ever occur in the eyeless specimens? Does a lens ever appear on the eyeless side in the single-eyed monsters? Finally do lenses ever arise in their usual lateral positions when the embryo has a ventro-median cyclopean eye? All of these propositions are affirmatively answered without an operation to injure in any way the ectoderm or tissues in the primary lens-forming region. It may be thought that the action of the chemical or anæsthetic is as severe as an operation but this is probably not true, as the embryos after being treated for the necessary time with magnesium, on being returned to sea-water develop, hatch and swim actively about, living in aquaria as long as I have tried to keep them, more than one month.

The experiments of the past summer have convinced the writer that his former idea that the eye defects are due to the anæsthetic properties of magnesium is correct; and there is no reason for believing that certain tissues usually between the eyes are entirely

absent. These results are given in full in another paper. The solutions employed act as anæsthetics preventing the usual outpushing of the optic vesicles from the brain to a greater or less degree, and give exactly the same condition so far as contact influence of the optic vesicle on the lens is concerned, as though the optic vesicle was cut away, without the disadvantages accompanying the operation.

The experiments with anæsthetics furnish a richness of material, hundreds of specimens being obtained, which one would be unable to duplicate from operations without spending days of tedious labor. The crystalline lenses may be seen with the binocular microscope as spherical refractive bodies in the living specimens. The experimenter in this way is enabled to select various condition for study.

The embryos were best preserved for histological study in picro-acetic though many fixitives gave good results. The lenses stain equally well in eosin or picric acid used as a counter stain after Mann's hæmatein or Delafield's hæmatoxylin.

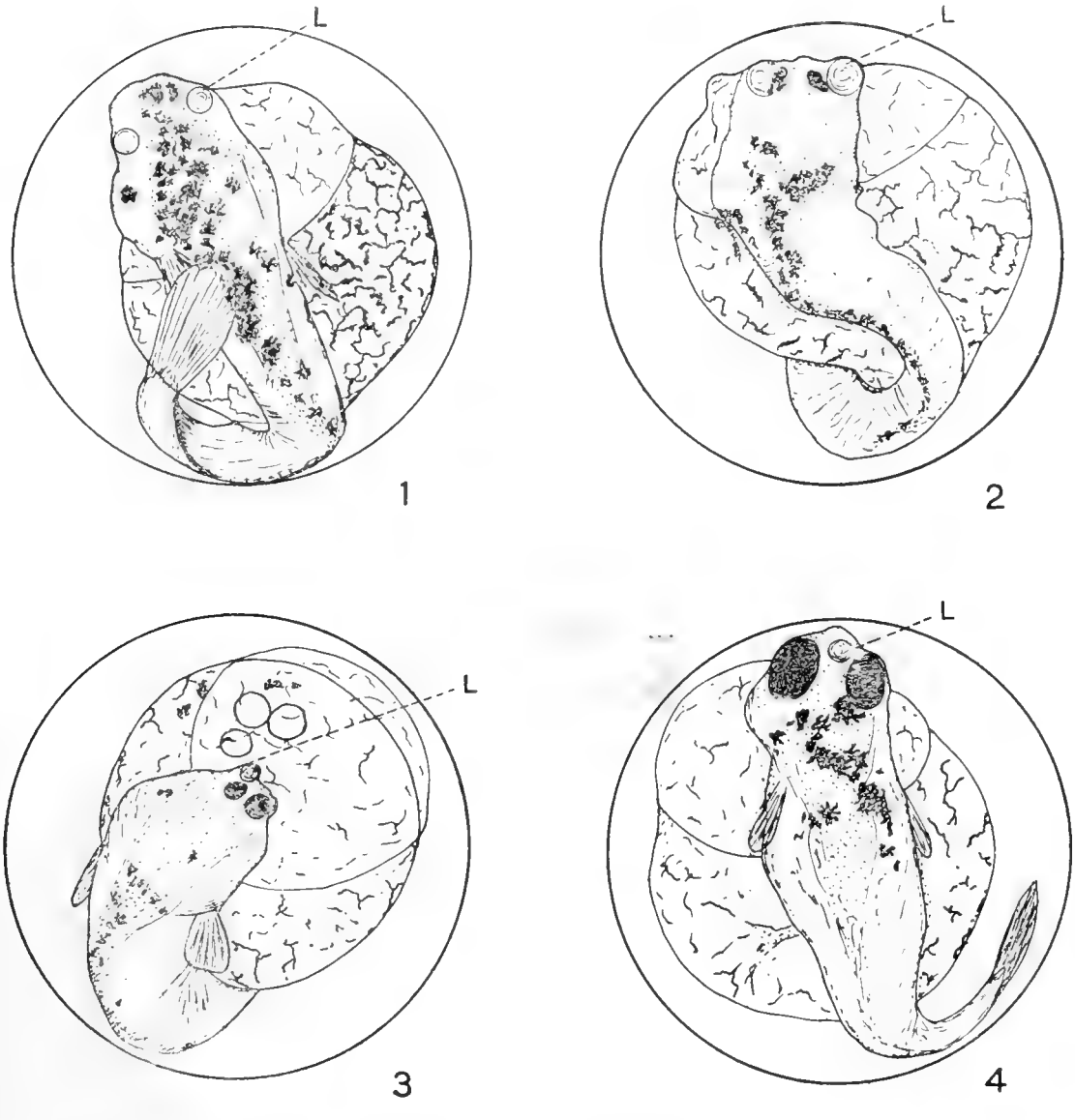
3 THE LENS IN LIVING EMBRYOS

The living embryos at various stages when examined with a binocular microscope show lenses isolated entirely from the optic vesicle or optic cup. Figure 1 illustrates an embryo eighteen days old that has no trace whatever of optic cups yet two perfectly developed transparent lenses occupy almost normal positions on the sides of the head. Sections of this specimen show the two lenses with well differentiated fibers, fig. 5 and plate II, fig. 3. Mesenchymatous tissue lies between the lenses and the brain. We must conclude that these lenses arose in lateral positions and continued development and differentiation with no direct influence whatever from either optic vesicle or the brain tissue. The possibility of optic cups having arisen and degenerated is entirely out of the question, since, in the first place, this has never been known to occur in any of the hundreds of Fundulus embryos that the writer has studied. In the second place, plate I, fig. 1, shows a lens arising from ectoderm on the eyeless side of

a seventy-six hour embryo; it is scarcely conceivable that an optic vesicle arose at about the thirty-fifth or fortieth hour, came in contact with the ectoderm and entirely disappeared, leaving no trace of itself by the seventy-sixth hour. Further, the lens in these cases must continue to develop and differentiate independently, which is contrary to the position held by Spemann ('05), Lewis ('07) and LeCron ('07) for the frog and salamander.

Fig. 2 shows a fish nineteen days old with two well formed lenses of normal size lying in contact with two small irregularly shaped masses of heavy black pigment. In sections, figs. 23 and 24, the lenses are found to be perfectly formed and the two dark spots are shown to be masses of choroid tissue or retinal pigment without a definite retina or other associated eye parts. The lenses may owe their existence to the choroid spots but the latter have failed to influence the size or manner of development of the former. If the origin of these lenses was due to the influence of the choroid areas we have a striking example of how very small an amount of optic tissue may call forth a lens. Many instances will be given to show that extremely small amounts of optic tissue touching the ectoderm will stimulate a lens to form, yet these cases will in no way weaken the evidence that lenses do at other times form entirely independently.

A fish is illustrated by fig. 3 with two small defective eyes deeply buried in the head. The right eye possesses a lens but the left faces ventrally into a mass of mesenchyme and is without a lens. In front of the left eye is shown a lens in an extremely anterior position but completely separated from the eye, and the concave surface of the cup is not directed towards this lens. Fig. 4 proves the case by showing two slightly small eyes each possessing its own lens, while somewhat in front and between the two eyes lies a perfectly isolated and independent lens. It is evident that this supernumerary lens is independently formed and not due to a stimulus from either of the eyes, since each has its own lens.



Camera Drawings of Living Fundulus Embryos, Magnified 16 Times

Fig. 1 An embryo eighteen days old. First thirty-six hours after fertilization were spent in 7 per cent alcohol in sea-water. No optic cups formed, yet two perfect crystalline lenses are shown in the sides of the head. See fig. 5 for a section of these lenses.

Fig. 2 An embryo nineteen days old, first thirty-six hours in 9 per cent alcohol. Two perfect lenses near poorly formed eyelike choroid spots. See figs. 23 and 24 for sections of these lenses and eye spots.

Fig. 3 An embryo of same age and lot as fig. 2; a free lens is seen in an extremely anterior position in front of an ill-formed eye. Lens. L.

Fig. 4 Another embryo of the same lot, showing a free lens between two somewhat defective eyes, each of which contains its own lens. Sections of this embryo, figs. 9 and 10, show also a second free lens which was hidden in the living specimen.

4 THE EMBRYOS STUDIED IN SECTION

a Is the Origin of the Lens from the Ectoderm Dependent upon a Contact Stimulus from the Optic Vesicle?

Spemann ('01) and Herbst ('01) first introduced the view that the lens originates from ectoderm only when a contact stimulus from the optic vesicle is present, although Spemann ('07) has since modified his opinion for one species of frog at least. Lewis ('04 and '07) has found this to be true in his experiments on frog tadpoles, and LeCron ('07) on salamanders. King ('05) claims, however, that such is not the case in her experiments and holds the view that the lens arises independently of the contact stimulus by the optic cup. Lewis ('07) has brought objections to the method employed by King and so criticises her results, but the writer believes her method has a real advantage in that she burnt out the optic vesicle areas of the still open medullary tube from the dorsal side and thus may not always have injured the lateral ectoderm of the future lens-forming region.

The writer's experiments on the fish embryos clearly demonstrate that the origin of the lens from the ectoderm may be entirely independent of the contact stimulus of the optic vesicle. He continues to use the expression "contact stimulus of the optic vesicle" since this is what has been deemed necessary for the origin of the lens, although he believes that a lens may arise without any stimulus whatever from the optic vesicle either by contact or from a distance.

In specimens lacking optic vesicles entirely it is difficult to imagine that tissues are present in the brain which possess the power to form substances characteristically formed by optic vesicles and that these substances diffuse until they reach the ectoderm and stimulate it to form a lens. In the case of isolated supernumerary lenses the optic cups possess lenses but still other lenses arise at a distance.

Again referring to fig. 1 of plate I, a section through the eye region of a seventy-six hour embryo, the ectoderm on the eyeless side is forming a lens which is somewhat slower in development

than the lens in the eye on the other side, yet this bud shows distinct lens character.

A perfect lens is seen in fig. 2, plate I, to be entirely separated from the brain and no optic cup exists. Fig. 13 shows a lens in a small choroid cup and a second free lens lying near. Fig 7 illustrates a similar case. Figs 8 and 12 show extremely anterior lenses in eyeless individuals and again fig. 5 and fig. 3, plate II, show two beautiful lateral lenses in another eyeless specimen. Fig. 6 shows a section with three well differentiated lenses all free from contact with an optic vesicle; a more posterior section of this embryo, fig. 27 shows a choroid cup deeply buried in brain tissue and without a lens. This cup does not come in contact with either of the three lenses shown in the more anterior sections.

Finally, a most remarkable case of supernumerary lenses is illustrated by figs. 9 and 10 and the outline fig. 11 shows the position of these lenses in the entire head (see also plate II, fig. 4). Two defective eyes each possessing a lens are shown in section, fig. 10, and a third lens lies between the eyes. In a more anterior region, fig. 9 and plate II, fig. 4, is found another section of this third lens, C, and a fourth additional protruding lens lies below it.

These cases might be enumerated and illustrated until they ran into the scores, but sufficient evidence has been given to prove that the crystalline lens in these embryos does *not* depend upon a contact stimulus of the optic vesicle for its origin from the ectoderm but originates independently.

b Is the Lens-Plate or Lens-Bud Capable of Differentiating into a Lens without Contact with the Optic Cup?

The above question is convincingly answered in the affirmative by the evidence given in the foregoing discussion. In the older embryos it is clearly shown that supernumerary lenses are as highly differentiated and as perfectly formed in all respects as are the lenses in the eyes. Eyeless individuals, as figs. 1 and 5 and plate II, fig. 3, indicate, may possess perfectly formed transparent lenses which appear in the living specimen as clear refractive bodies.

Sections of Well Differentiated Free Lenses

Fig. 5 A section of two free lateral lenses in the nineteen day embryo shown by fig. 1.; no trace of optic cups exists. This embryo has two ears of unequal size.

Fig. 6 Section of the head of nineteen day embryo, first thirty-six hours in 9 per cent alcohol. Three free lenses, A. B. and C are shown, a defective optic cup completely separated from the lenses is deeply buried in the head tissues of a more posterior region, see fig. 27.

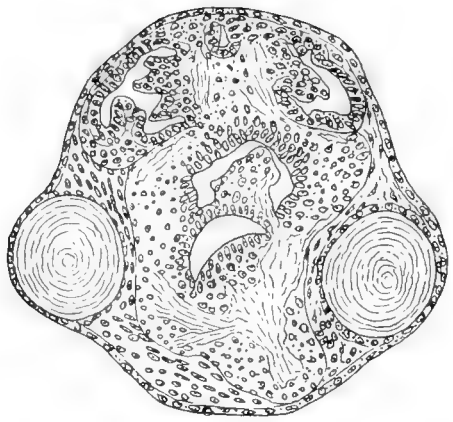
Fig. 7 Section of the anterior tip of the head of a nineteen day embryo from 9 per cent alcohol. The upper right lens protrudes from a defective eye shown in more posterior sections, while the lower lens, F., is free, being in no way associated with an eye.

Fig. 8 A somewhat sagittal section of a similarly treated embryo of same age, showing another free lens, p, a pigment spot.

Fig. 9 An anterior and fig. 10 a more posterior section through the eye region of a nineteen day embryo treated with alcohol. The diagram, fig. 11, shows the plane of both sections. Fig. 4 shows the same embryo from life; the eyes in the sections are reversed by the microscope. Two optic cups are present each with a lens, A. and D, while two other perfectly differentiated lenses, B and C, are not connected with an optic part.

Fig. 12 A section through the anterior tip of a pointed-headed eyeless embryo nineteen days old. The lens is well differentiated; the ears in this specimen are scarcely formed.

Fig. 13 A section of a nineteen day embryo showing a small defective choroid cup with a lens, and a second accessory lens is near by.



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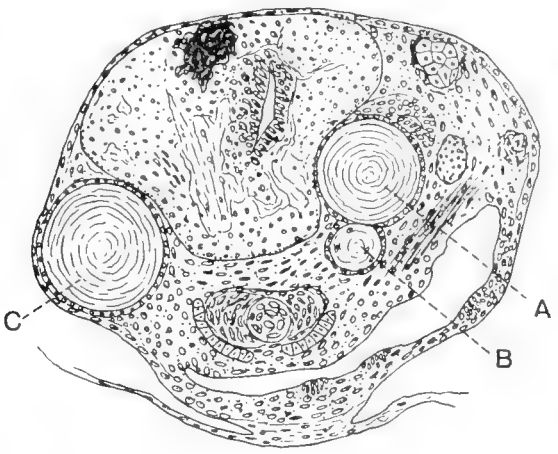

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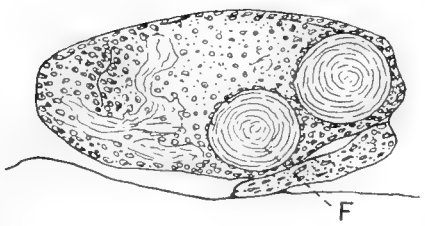

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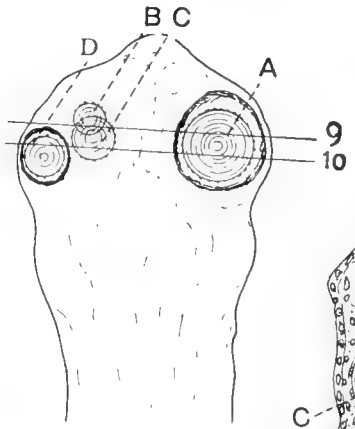

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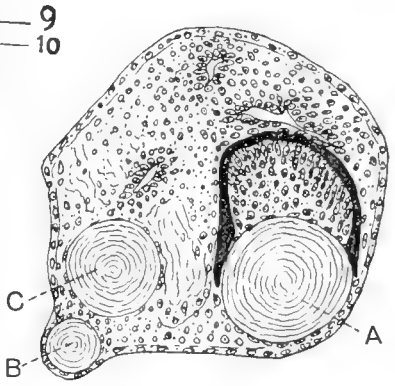

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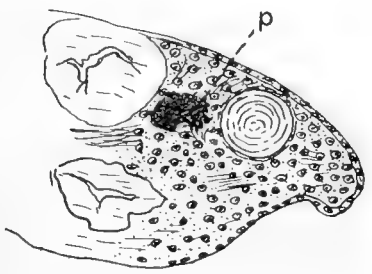

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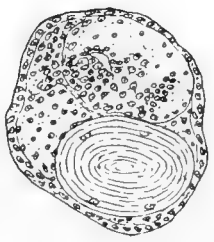

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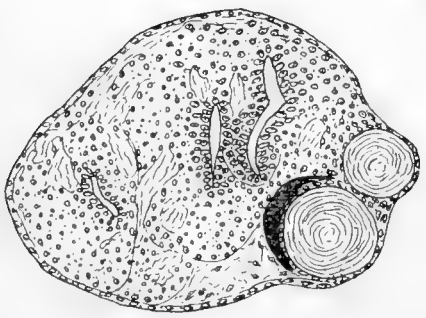

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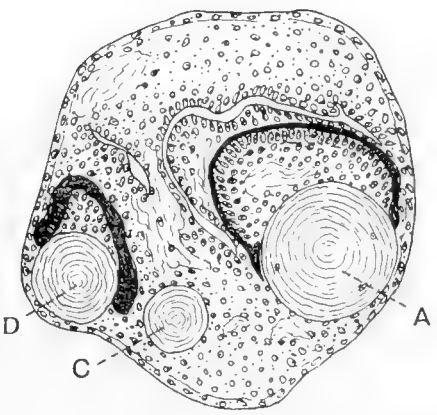

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The possibility of the action of some substance given off by a distant optic cup is entirely aside, since other experimenters have claimed that when the optic vesicle or cup is in any way separated from the lens the latter organ begins to degenerate and usually disappears.

Figs. 6, 7, 9, 10, 12, 13 and 21 and plate I, fig. 2, plate II, figs. 3 and 4, all go to show that in the fish embryo the lens-plate or lens-bud is capable of self-differentiation, finally forming a perfectly transparent refractive body even though completely isolated from any other eye-like structure.

c Does the Size of the Optic Cup Regulate the Size or Shape of its Associated Lens?

Lewis ('07) has stated in his more recent paper on the lens that, "The lens is neither self originating nor self differentiating, but is dependent for its origin, its size, its differentiation and its growth on the influence of the eye." The writer had also independently been led to think from his first experimental study of cyclopia ('07), which was based on a limited supply of material, that the size of the lens varied directly with the size of the optic cup. He is now able to show that while normlly the lens and optic cup are properly adjusted as to size this is not by any means constantly true of ill-formed eyes. Here the size and also the shape of the lens is often greatly out of accord with that of the optic cup. In normal eyes the optic cup has a definite size and so does the lens. The sizes accord, yet this may be incidental or entirely without correlation, as is suggested by the fact that optic cups of unusual shape and size are not able to regulate the development of the lens so as to adjust it to their strange proportions.

Many of the illustrations of cyclopean eyes given in the writer's recent paper ('09) show misfits between the cups and lenses. Remarkable cases are also shown by figs. 9 and 10 and plate II, figs. 4 and 5, in which the lenses are clearly too large for the associated cups. Fig. 15 shows the two components of an incomplete cyclopean eye with one normally proportioned lens between them. This lens is scarcely large enough to function with the unusually

wide double cup. In fig. 20 is seen a somewhat similar double cup with an elongated and slightly constricted lens; fig. 18 also shows one half of a double cyclopean eye with a double lens, the other half eye is in a more posterior section. In figs. 14 and 17, on the other hand, we find illustrated double lenses in one of two closely approximated eyes. Fig. 14 also shows a tiny additional lens still further within the same cup which possesses the large double lens. Fig. 16 shows the extreme anterior tip of a cyclopean eye with two minute lenses protruding from it. This section is only fifty micromillimeters from the anterior end of the head. Numerous other examples of misfitting lenses might easily be given.

These facts force us to conclude that the size of the optic cup does not fully regulate either the size or shape of the associated lens. It is, therefore, evident that the usual harmonious adjustment between the optic cup and the lens may not be so entirely due to a dominating influence of the optic cup on the lens as one might be led to believe from previous contributions to the subject. That some influence or interaction exists, the writer does not deny, and will show in the following parts of this paper the remarkable ability possessed by the optic vesicle to obtain a lens from any part of the ectoderm with which it may come in contact.

d Is the Optic Vesicle, Normal or Defective, always Capable of Stimulating Lens-Formation from the Ectoderm at Some Stage of its Development?

Of all the embryos which the writer has examined not one failed to have a lens in a normal optic cup when the cup came in contact with the ectoderm. If, however, the cup fails to reach the outer body wall, although it may possess well differentiated retinal layers and other parts, it is invariably without a lens, fig. 26. The convex side of the choroid coat or pigment layer of the retina does not cause a lens to arise even though it be closely applied to the ectoderm, as is shown by fig. 26 and many other illustrations in which the choroid touches the body wall.

Defective optic cups when deeply buried and separated from

Ill-Adjustment of Optic Cups and Lenses in Thirteen Day Fish Embryos.

Fig. 14 A section of an embryo treated with 5 per cent alcohol. Both optic cups are defective, the left one contains a spherical lens, while the right cup has a large lens, in shape a constricted oval, and a tiny spherical lens placed further within the same cup. E, ear.

Fig. 15 A poorly formed eye of the incomplete cylopean type. Each component faces in a ventro-median direction and a spherical lens lies between them An ear, E. is shown on the side of the better component, the other ear is absent.

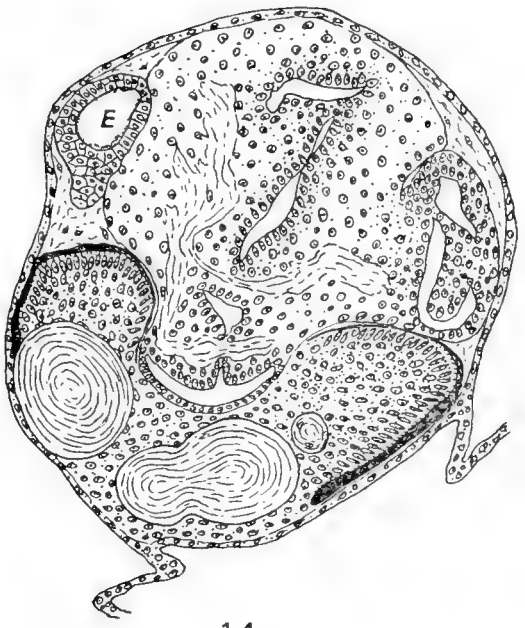
Fig. 16 A section 50 microns from the anterior tip of an embryo. The anterior border of a cyclopean eye is shown with two protruding lenses of very minute size. ec, ectoderm.

Fig. 17 Section through the anterior tips of two small *closely neighboring eyes*, whose median planes are in more posterior sections. The smaller eye has a protruding double lens. This embryo possesses only one ear located on the side with the better eye.

Fig. 18 Part of an incomplete cyclopean eye (other half in more posterior sections) containing a double lens.

Fig. 19 Two *adjacent eyes* facing ventro-medianly with two lenses.

Fig. 20 An ovoid lens in an incomplete cyclopean eye.



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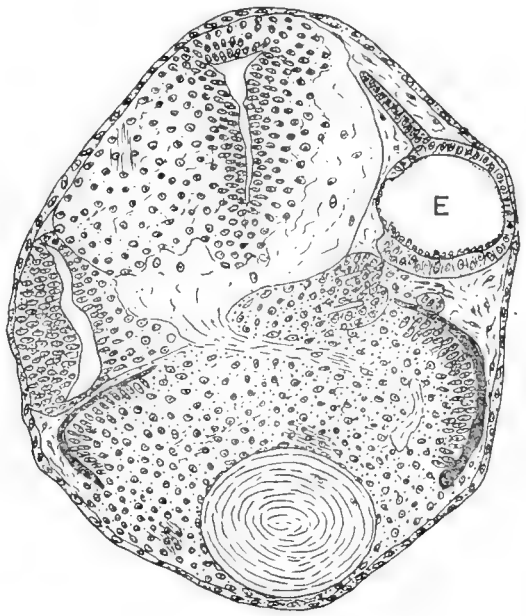

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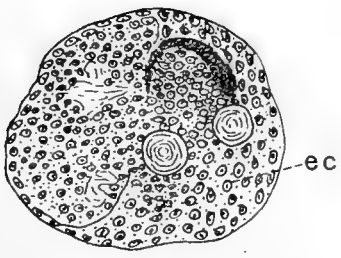

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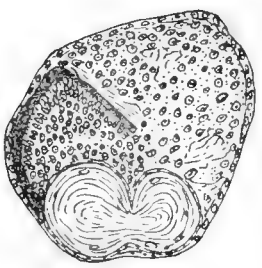

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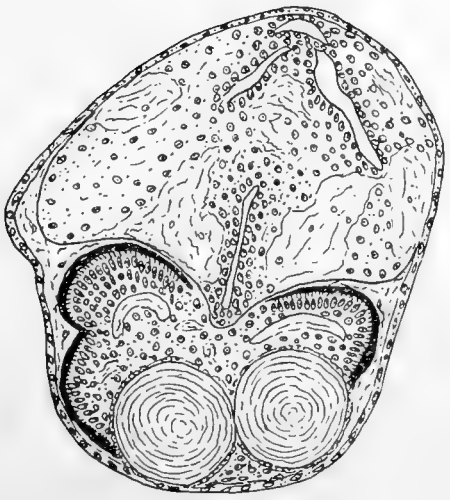

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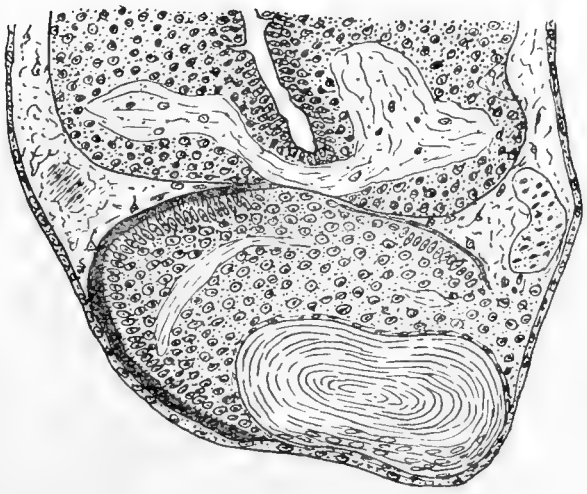

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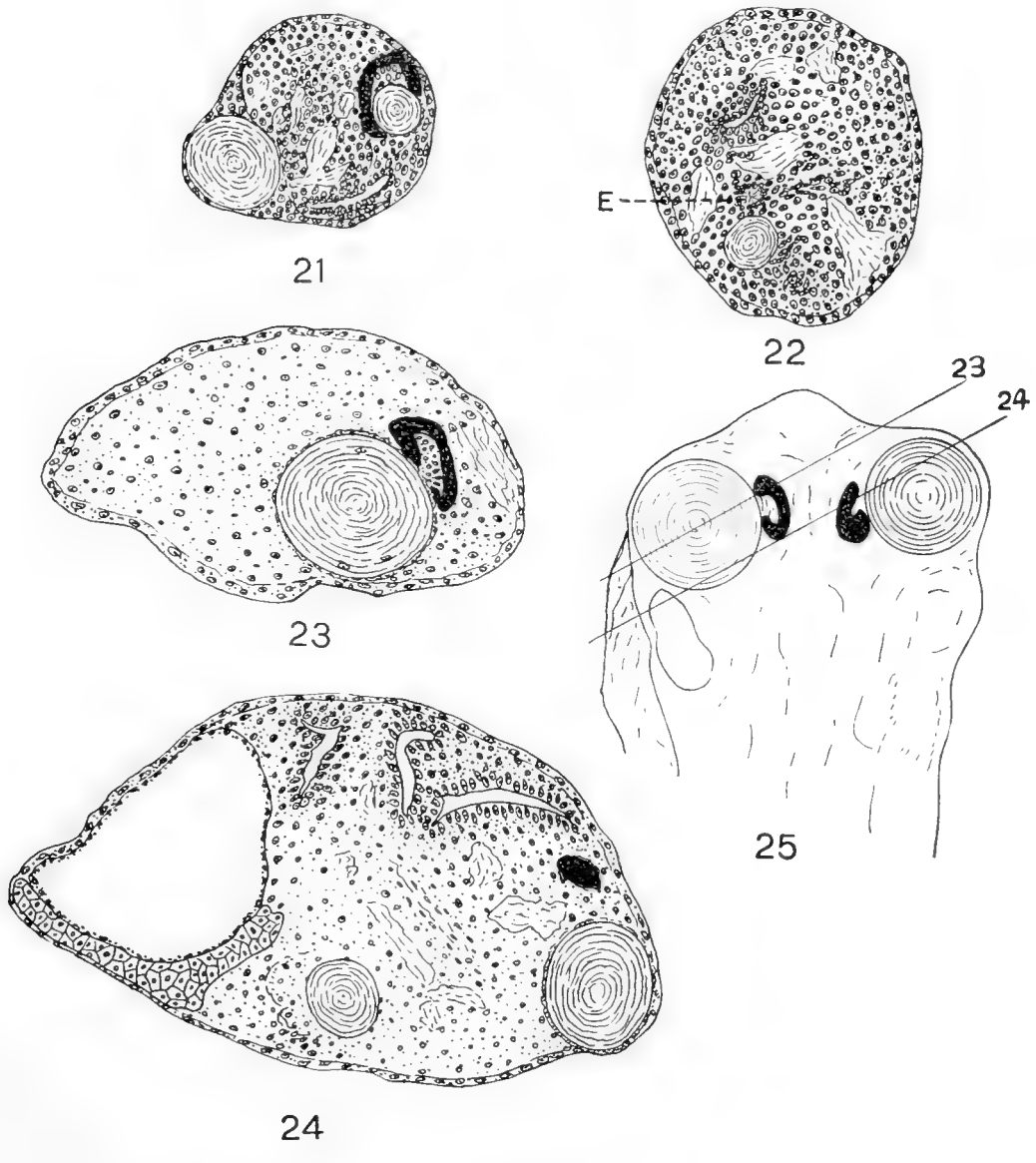
the ectoderm also lack a lens, as is illustrated on the left of figs. 27 and 28. On the other hand, it is remarkable how small and ill-formed an optic cup-like structure has the power of stimulating a lens to arise from the ectoderm. Figs. 2, 23 and 24 and plate II, fig. 5, show small choroid cups with no retinal differentiation whatever, yet closely associated with perfectly formed lenses. In fig. 21 is seen an extremely defective cup with a small lens; a larger independent lens is shown on the eyeless side. Fig. 22 illustrates an extremely insignificant eye-like body buried within the brain, yet close by is a small crystalline lens; these are the only eye parts found in this embryo. In fig. 19, a section through the eye of an incomplete cyclops, each component of the eye has a lens, while in fig. 20 the eye components are closer together and in fig. 15 further apart, yet each of these possesses only a single lens, although it is elongate in fig. 20. It is difficult to say why such eyes occasionally possess two lenses. After an examination of a large number of such eyes no general rule is found. It may be due in some way to the manner in which the optic cup periphery meets the ectoderm, whether as a circle, an oval or at times a much constricted oval so that two areas of ectoderm are separately stimulated to form lenses.

This consideration forces the conclusion that an optic cup at some stage in its development, whether normal or defective, invariably possesses the power to stimulate lens-formation from the ectoderm with which it comes in contact.

e May the Optic Vesicle Cause Lens-Formation from Ectoderm Other than that which Normally Forms a Lens?

This question is answered by the cyclopean monsters. It is scarcely conceivable that the ectoderm which would normally lie over the lateral eyes has the power to migrate, or follow the optic vesicle so exactly as always to lie just over the vesicle wherever it may chance to develop. Many embryos have eyes in unusually anterior positions and derive their lenses from anterior ectoderm, while others possess ventral eyes with lenses derived from ventral ectoderm. It occurs in a few cases, as the writer previously



Extremely Defective Optic Cup-Like Bodies Associated with Perfect Lenses

Fig. 21 An anterior section, as is indicated by its size, of a nineteen-day embryo. A free lens is shown on the left while on the right a very small defective eye contains a small lens.

Fig. 22 A small lens near a vesicle-like structure, E, in the brain which might represent an abortive optic body.

Figs. 23 and 24 Sections through the embryo shown in fig. 2. In the diagram, fig. 25, is shown approximately the planes of the sections. The large well-formed lenses are associated with defective optic cups which lack any sign of differentiation.

recorded ('09), that free lenses arise in their usual lateral positions while the cyclopean eye possesses its lens of anterior origin.

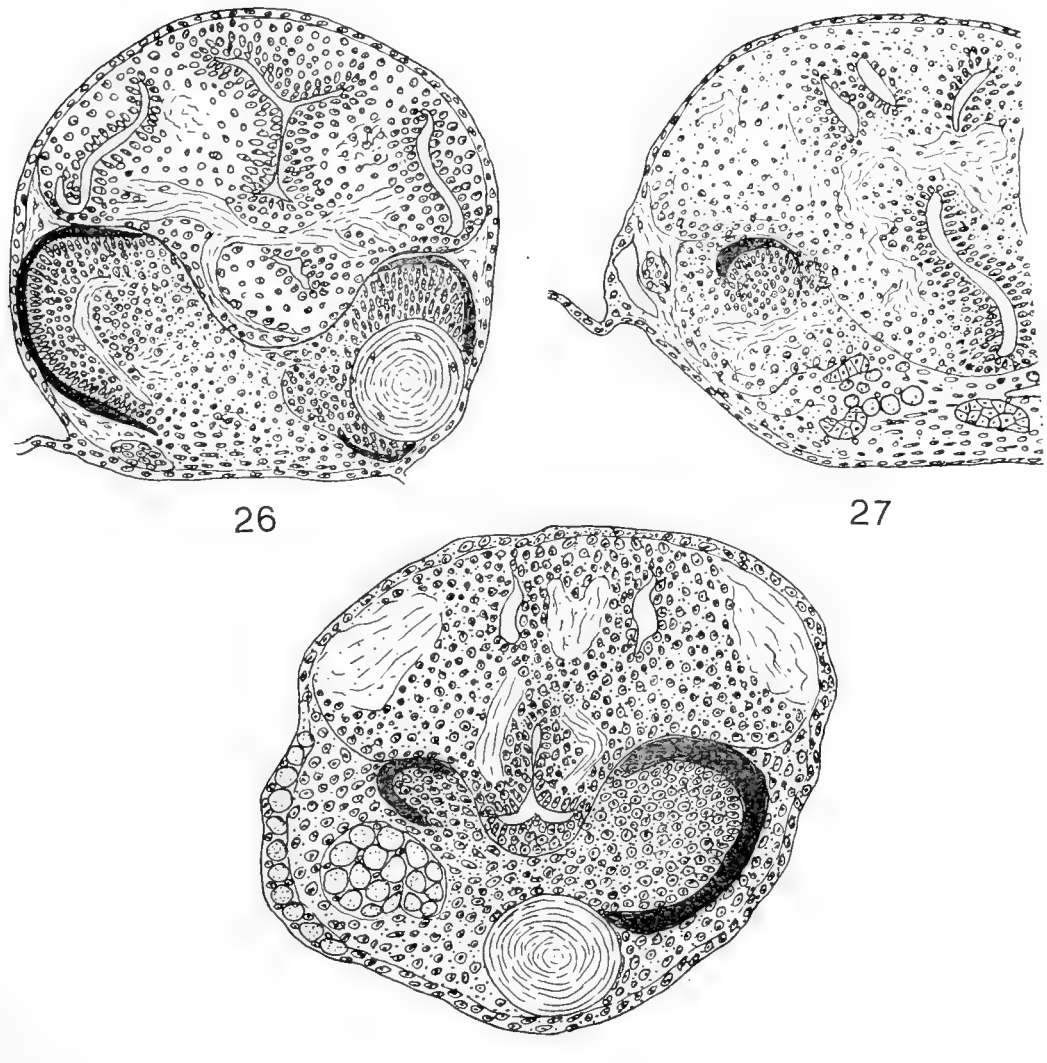
In the fish embryo the optic vesicle may cause a lens to form from ectoderm far removed from the usual lens-forming area, and rarely in such cases it happens that free lenses may also arise from the usual lens-forming region.

f Does a Deeply Buried Eye have the Power to Regenerate or Form A Lens from its own Tissues?

It has been shown by many experimenters Colucci ('91), Wolff ('95 and '01), Müller ('96) and Fischel ('02), that the salamander's eye regenerates a new lens from the posterior surface of the iris when the old lens is removed, or as Fischel found, if the old lens be merely pushed back out of its usual place in the eye. When the iris was injured in two places during the extirpation of the lens, two lenses arose within the single eye, one growing from each injured area of the iris. It has also been found that a fish's eye would regenerate a lens under certain conditions: when the fish is young and when a sufficiently long time is allowed for the lens to regenerate.

Lewis ('04) finds that the deeply buried eyes in Rana palustris which fail to come in contact with the ectoderm are unable to form lenses from their own tissues. While on the other hand he states that in a second species, Rana sylvatica, the optic cup readily gives rise to a lens from its own tissues if prevented from stimulating the formation of a lens from the ectoderm of the body wall.

The fish embryos which are now being considered, act in a similar manner to Rana palustris and are unable to form lenses from the tissues of their optic cups. Whenever the optic cup is deeply buried and fails to reach the ectoderm it also fails to possess a lens, as is illustrated on the left side of figs. 26, 27 and 28. In this connection it may be mentioned that Morgan was unable to obtain the regeneration of a lens in adult specimens of Fundulus, although as mentioned above, lenses do regenerate in the eyes of another species of fish.



Deeply Buried Eyes without Lenses

Fig. 26. A section of a thirteen day embryo which was treated with alcohol. One eye faces outward and contains a lens, while the other faces in toward the median plane of the head and is without a lens, probably never having come in contact with ectoderm.

Fig. 27 A section of a nineteen day embryo more posterior in the same series than section, fig. 6. The deeply buried defective eye has no lens.

Fig. 28 Section of thirteen day embryo after treatment with alcohol. Section passes in front of the median plane of the right eye which is well differentiated and contains a lens. The other eye is small and scarcely differentiated, buried in the tissue below the brain and contains no lens. A normal ear exists on the side with the large eye and a small defective ear is on the other side in more posterior sections.

5 DISCUSSION OF PREVIOUS OBSERVATIONS AND EXPERIMENTS

Before drawing final conclusions from the cases discussed above it may be well briefly to review the modern work and experiments which have been directed towards a solution of the so-called lens problem.

Rabl ('98) in his study of the structure and development of the lens, found in one case, at least, that a lens-like body was present, although far removed from the optic vesicle. This observation has been criticised by Lewis, who suggested that the ectoderm with the newly forming lens had shifted away from the small optic vesicle. Again, Lewis states that Rabl's case, and also a case to be considered below that was shown by Mencl, prove neither one side nor the other, since the experimental evidence is all directly for the idea that a lens will not arise from the skin without the stimulus of the optic vesicle. At the present time, however, the experimental evidence points in an opposite direction, and the cases of Rabl and Mencl can scarcely be disposed of in so brief a manner. On the contrary Rabl's example must be considered the initial illustration of the origin of an early lens without a stimulation from the optic vesicle.

Following this single case a strong tide turned towards the idea of the dependent origin and development of the optic lens. Herbst's paper ('01) on "Die formative Reize in der thierischen Ontogenese" and Spemann's pioneer experiments ('01) on the development of the lens seemed most convincing evidence in favor of a correlation in development between the optic vesicle and the lens, a correlation in which the latter played a dependent rôle. Herbst claimed the optic lens to be a "Thigmomorphose" originating only by a contact between the optic vesicle and the epidermis. His reason for such a position being that in the case of cyclopean monsters the median optic vesicle always derives a lens from the overlying ectoderm, while no lenses arise in the usual lateral positions. "If the lens is an independent organ why does it not arise in the lateral eye region of cyclopean monsters?" In former experiments the writer ('09) produced just such a case as Herbst thought necessary to show the independent origin of

lenses, that is, a cyclopean monster with lenses in the usual lateral positions.

After a study of a great number of such monsters I am now able to reaffirm that free lenses do occasionally arise in the lateral eye regions. Herbst's clever argument based on pathological embryos is, therefore, rendered invalid.

Spemann ('01) was the first to clearly attack the problem experimentally. He injured or destroyed the optic vesicles of Triton embryos by means of hot needles and electric cauterizers. The method was not altogether satisfactory since such an operation often injures much of the surrounding tissue, yet Spemann's results were of the highest value, and stimulated an active interest on the part of many experimenters. His conclusions furnished strong support for Herbst's idea of the dependent origin of the lens. He found that whenever the optic vesicle was so injured that it failed to come in contact with the head ectoderm, the ectoderm failed to form a lens. The lens was, therefore, dependent for its origin on a contact stimulus of the optic vesicle upon the ectoderm. Later Spemann ('05) also concluded that the lens was not self differentiating but that a durable contact stimulus of the optic cup was necessary for it to form lens fibers. Thus the "Herbst-Spemann theory of dependent lens formation," as Mencl has termed it, was developed. We shall see below, since it seems best to consider these papers in a more or less chronological order, that Spemann's own later work has helped materially to overthrow this theory.

Barfurth ('02) also operated with a hot needle to destroy the lens anlage and the optic cup. The embryos were examined after five or six days. One specimen showed on one side a poorly regenerated optic cup that did not come in contact with the ectoderm and yet a lens still connected with the ectoderm was present on this side. Barfurth was inclined to accept the Herbst-Spemann idea of lens formation and so attempted to harmonize his case with the theory as follows. Some sections of the embryo, 9 to 12, showed the optic vesicle lying very near the ectoderm but not in contact with it. Barfurth supposes that at an earlier period in development it may have been in direct contact with

ectoderm and at such a time stimulated the lens to arise. This is merely a conjecture and the more recent experiments of King ('05) and Spemann ('07) on the frog, and the writer's on the fish, would warrant equally well an interpretation of independent origin for this lens.

Returning to observations on monsters we may consider the case reported by Mencl ('03) which has called forth so many explanations and criticisms. This report described the existence of an independent lens on each side of the head in an anadidymus embryo of Salmo salar which he had possessed since 1899, his interest in it being aroused by the contributions on the dependent origin of the lens. One of the lenses in this monster was closely applied to the brain wall, and in fact lay in a depression in the side of the brain; the other lens, however, was completely separated from the brain by mesenchyme. Spemann and Lewis have tried to explain this case by assuming the existence in the brain wall of some optic vesicle tissue or substance which when brought in contact with the head ectoderm possessed the power to cause the formation of a lens. There was absolutely no evidence of optic tissue in this brain wall and further, the explanation could apply only to one of the lenses, though it scarcely explains the origin of either. The second lens, entirely free and with mesenchymatous tissue separating it from the brain wall, was as perfect, though not so large, as the other which lay against the brain. This lens probably arose and developed freely, just as did so many lenses in the fish embryos the writer has studied.

Mencl ('08) has more recently obtained other anadidymus Salmo embryos and confirms his former observations, finding that in such monsters free lenses are present in 25 per cent of the cases.

Gemmill ('06) has also recorded the frequent occurrence of free lenses in the heads of monster trout embryos.

Schaper ('04) in considering a case of a typical lens development comes to the theoretical conclusion that the lens is by nature a primitive sense body similar to the sinnesknospe of Amblystoma, and has secondarily taken on its present function. It must, therefore, arise independently of the optic vesicle, with which it is only recently associated. Such speculation

is not entirely out of accord with the present facts of lens formation.

Lewis thinks that the free rudimentary lenses in Schaper's experiments were undoubtedly caused by the shifting of the ectoderm and lens away from the optic vesicle. The writer is unable to see any reason why such a shifting is supposed to have taken place. On the contrary, the facts seem to lend themselves more readily to the interpretation of a free origin of the lens.

The experiments of Lewis ('04) seemed to show convincingly that the lens was dependent for its origin and development upon a contact stimulus of the optic vesicle on the ectoderm. Lewis devised a method far more refined than any previously used in similar experiments. He operated on tadpoles under a binocular microscope with needles and small scissors and was able to cut the ectoderm and fold it forward so as to expose the brain and early optic vesicle, which could now be cut away. The ectoderm was then folded back in place. From these experiments the following are some of the conclusions which were drawn:

Neither a lens nor a trace of a lens will originate from the ectoderm which normally gives rise to one, if the contact of the optic vesicle with the skin is prevented.

There is no predetermined area of the ectoderm which must be stimulated in order that a lens may arise. Various parts of the skin when stimulated by optic cup contact may and do give rise to a lens.

In normal development the lens is dependent for its origin and differentiation on the contact influence or stimulation of the optic vesicle on the ectoderm.

The conclusions are clearly stated and the experimental method employed is most skillful, but not entirely free from objection. The conclusion regarding the entire dependence of the lens on an optic vesicle stimulus will not, the writer believes, be supported by future experiments, and indeed at the present time such an idea has a vast amount of evidence against it, unless the particular species experimented with shows a specific action in lens formation. The later work of Spemann ('07) which contradicts his former conclusion shows that in some species of frogs the lens

may arise and differentiate independently of the optic vesicle stimulus. In these experiments it must be noted that Spemann operated with glass needles to remove the optic vesicle regions from the open medullary plate. Such an operation does not injure the lens-forming region of the ectoderm as Lewis' experiment does and it is on this account, the writer believes, that the free lenses arise.

The tendency of the ectoderm to form a lens independently is so delicately adjusted that a very slight injury or disturbance may suppress it, yet the same ectoderm may still have the power to form a lens in response to the stronger stimulus of the optic vesicle. So in experiments where the ectoderm has been cut or injured it loses the power to form free lenses even though the optic vesicle can stimulate a lens to arise from it. When the ectoderm is uninjured, as in some of King's specimens, Spemann's ('07), and the writer's, then free lenses do occur.

We may imagine the lens to represent an ectodermal organ formerly of independent importance. However, it has now become so closely associated with the nervous portions of the eye that it arises whenever such a part meets the ectoderm, yet the lens retains to a feeble degree its impulse to arise independently of other eye parts. When it has once arisen it is perfectly capable of differentiation. Future experiments of removing the optic vesicle without injuring the ectoderm will probably demonstrate further this tendency of the lens to arise independently, just as Mencl's observations and the writer's experiments show for the fish, and many of the experiments mentioned show for amphibians.

King (05) states that she had begun her series of experiments in 1900. She destroyed the optic vesicles from the forebrain region in the closing medullary tube with hot needles. Many of the embryos died as a result of the operation. The conclusions reached by King are entirely opposed to those of Lewis and Spemann's earlier results regarding the dependent origin of the lens. King found that in some of the embryos a lens-like body arose from the ectoderm on the side with an injured or unregenerated optic cup which did not come in contact with either the ectoderm or the lens-like body. Some of the specimens show a very suggestive

lens-like structure which is entirely free from any contact with an optic vesicle. The inaccuracy of the operation may account for these lenses, on the ground that in exceptional cases the outer ectoderm of the lens region was too far distant at the early stage of development to have been injured. This is probable when it is noted that the operation did not enter through the lateral ectoderm, but through the partly open medullary tube from above. The present evidence also goes to strengthen King's position on the subject.

Spemann ('05), Lewis ('07) and LeCron ('07) have all claimed that the lens after its origin, was not self differentiating but depended upon a durable contact with the optic cup for its future development. All of the writer's experiments and the observations of Mencl are clearly contradictory to such a view, and Spemann ('07) has more recently found the lens to be self-differentiating in Rana esculenta.

In a former paper the writer ('07) described the development of the lens in the blind Myxinoid, Bdellostoma stouti. At that time he presumed that this animal furnished support for the experimental evidence that the lens was not self-differentiating. In Bdellostoma embryos the optic vesicle is well formed at first and reaches out from the brain to the ectoderm; at this time the ectoderm forms a localized thickening suggesting a lens-plate. The optic vesicle then ceases to maintain its progressive development and loses connection with the ectoderm, finally giving rise to the poorly formed optic cup of the adult. The early lens thickening of the ectoderm degenerates and is entirely absent from older embryos. The writer suggested that this degeneration was due to the loss of contact with the optic vesicle, since such reasoning seemed correct in the light of the experiments up to that time. He has entirely changed his view, however, regarding the matter of the lens in Bdellostoma and now believes that it represents a rudimentary organ which has been lost in the adult and appears only for a short time during embryonic life. As Eigenmann ('01) records of Amblyopsis, the lenses of other blind fishes appear at certain stages in the developing embryo and then degenerate. A case not so far advanced in the degeneration of the eye and lens is

that of the Florida burrowing lizzard, Rhineura. In the adults of Rhineura, Eigenmann finds lenses sometimes present and sometimes absent and when present they are very variable. So many examples are known of the traces of rudimentary organs in the embryo which are entirely lacking in the adult that the above cases of the lens are not at all strange and at present it seems that this is the most satisfactory explanation of their behavior.

The more recent experiments of Spemann ('07) so frequently referred to, may be briefly described. Embryos of Rana esculenta were operated upon with glass needles so as to remove the optic vesicle areas from the open medullary fold (his former experiments were made with hot needles, which doubtless injured more of the surrounding tissues).

After allowing these embryos to develop for several days and then studying them in section it was found that in one case on the side of the head lacking an optic vesicle a true lens-bud was still in connection with the ectoderm. In an older embryo a free lens vesicle was found, and finally, in a still older specimen a lens was buried in connective tissue on the eyeless side of the head yet it possessed fully formed lens-fibers. Thus, Spemann, five years after his first paper on the dependent origin of the lens, now concludes that the lens in Rana esculenta is self-originating and self-differentiating. He also accepts Mencl's case of the independent lens in Salmo salar.

We have thus seen in a rather cursory survey how the lens problem has arisen and how experiments have built up first one side of the question and then the other, and many may also feel that the final word is yet to be added. Nevertheless, it is true that at this stage of the investigation it has been clearly demonstrated in several groups of animals that the ectoderm possesses the power independently to originate a lens which subsequently develops into the transparent refractive organ usually found within the mature eye. The manner of origin and differentiation of the lens may easily differ in different animal species, and the statement would not be warranted that all of these facts apply to animals in general.

The lens is self-originating and self-differentiating yet it is more

emphatically proven that the optic vesicle or optic cup has the power without exception to cause a lens to form from the ectoderm with which it comes in contact, even though this ectoderm may be far distant from the usual lens-forming area. There is probably no strictly limited region of ectoderm from which the optic vesicle must stimulate lens-formation. The self-originating lenses, however, have invariably occurred in the head region, though not always in their usual lateral positions. It would, therefore, seem that the ectoderm of the head is more predisposed to the formation of lenses than that of the other body regions.

6 SUMMARY AND CONCLUSIONS

1 When the developing eggs of Fundulus heteroclitus are subjected to the action of Mg salts, alcohol, or other anæsthetic agents, the normal outgrowth of the optic vesicles is generally inhibited. Embryos are obtained either entirely without optic cups, with small deeply buried eyes, with only a single eye on one side of the head or, finally, with a median more or less double cyclopean eye. These specimens furnish exceptional material for a study of the relationship between the development of the optic vesicle and the optic lens. The embryos with the nervous eye parts entirely lacking are similar to specimens with the optic vesicles mechanically cut out of the brain. At the same time, the injury to the ectoderm which usually results from the operation is avoided, and this is a most important advantage. When Spemann ('07) operated on Rana esculenta embryos with open medullary plates he was able to remove the optic vesicle areas from the future inner side of the tube without injuring the ectoderm of the lens-forming region and in such cases independent lenses arose on the eyeless side. King ('05) obtained free lenses in a somewhat similar experiment. Lewis' embryos might possibly respond in a like manner if operated upon in this fashion. The optic vesicle may stimulate a lens from slightly injured ectoderm, but the free origin of lenses is a more delicate process and one more easily interfered with.

2 The crystalline lens may originate from ectoderm without any direct stimulus whatever from an optic vesicle or cup. These self-originating lenses arise from regions of ectoderm that are not in contact with either optic vesicle, the brain wall, or any nervous or sensory organ of the individual (see plate I).

3 The lens-plate or lens-bud is capable of perfect self-differentiation. No contact at any time with an optic vesicle or cup, is necessary. These lenses finally become typical transparent refractive bodies exactly similar in histological structure to that in the normal eye (see plate II).

4 The size and shape of the lens is not entirely controlled by the associated optic cup. Lenses may be abnormally small for the size of the cup, or entirely too large, so that they protrude; or, finally, peculiarly shaped oval or centrally constricted lenses may occur in more or less ordinarily shaped optic cups. The lens is by no means always adjusted to the structure of the optic cup as has been claimed by some observers.

5 An optic vesicle or cup is invariably capable at some stage of its development of stimulating the formation of a lens from the ectoderm with which it comes in contact. It is remarkable how extremely small an amount of optic tissue is capable of stimulating lens formation from the ectoderm (plate II, fig. 5).

6 The optic vesicle may stimulate a lens to form from regions of the ectoderm other than that which usually forms a lens. This is shown by the fact that a median cyclopean eye always stimulates a lens to form from the overlying ectoderm. It is scarcely possible that the lateral normal lens-forming ectoderm could follow the cyclopean optic cup to the many strange situations it finally reaches.

7 The ectoderm of the head region is more disposed to the formation of lenses than that of other parts of the body, since the free lenses invariably occur in this region.

8 A deeply buried optic vesicle or cup may fail to come in contact with the ectoderm; in such cases it lacks a lens. The tissues of the embryonic cup itself are unable to form or regenerate a lens. This is not true in all embryos, as Lewis has shown for one species of frog.

9 The optic lens may be looked upon as a once independent organ (possibly sensory or perhaps an organ for focusing light on the brain wall, before the vertebrate eye had arisen) which has become so closely associated with the nervous elements of the eye that it has to some extent lost its tendency to arise independently, although still capable of doing so under certain conditions. The lens now arises much more readily in response to a stimulus from the optic vesicle, a correlated adjustment which insures the almost perfect normal accord between the optic cup and the lens. In experiments on the origin of the lens one must guard against disturbing the ectoderm from which an independent lens might arise. Although an optic vesicle might have the power to stimulate a lens from injured ectoderm, the innate tendency of the ectoderm to form a lens is a more delicate impulse and may be suppressed by a slight injury or disturbance of the ectoderm during the operation.

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Explanation of Plates I and II

Fig. 1 A photograph of a section of a seventy-six hour Fundulus embryo; the brain is almost bilateral and perfect, with a well formed left optic cup, no indication of the right cup exists, yet the ectoderm on that side has formed a well pronounced lens-bud, L.

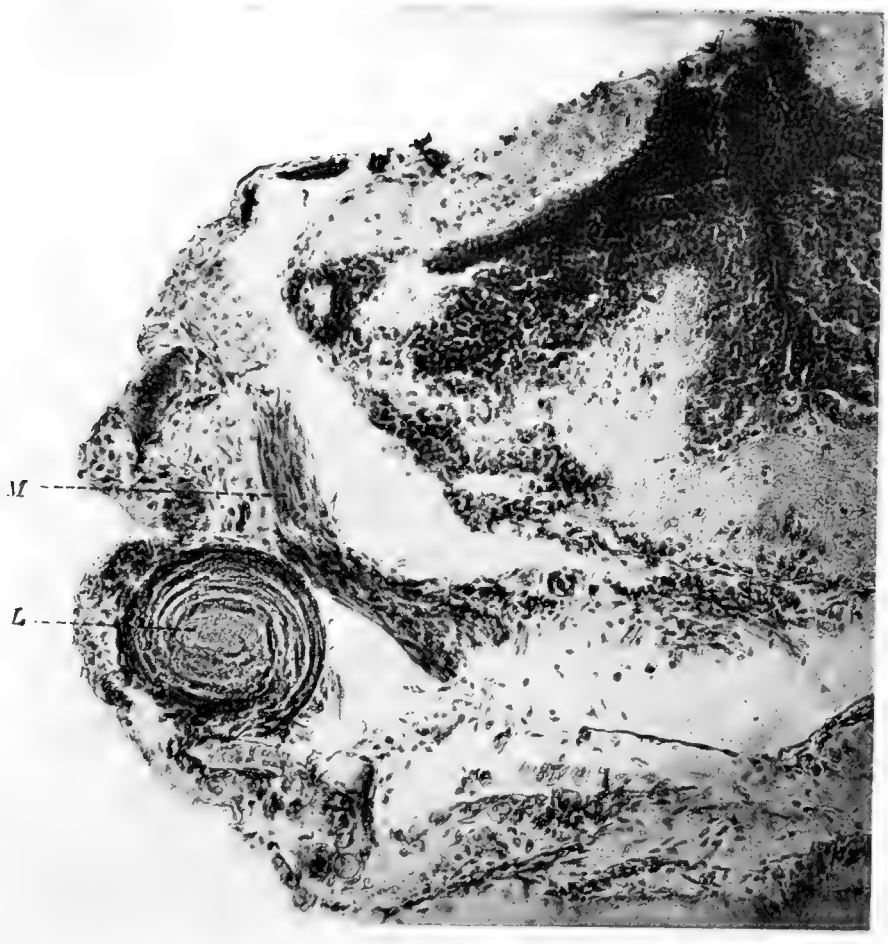
Fig. 2 A section through the eye region of a thirty day embryo, there is no optic cup in the specimen. A perfect lens is in the usual lateral position, L, a band of muscle, M, lies between it and the brain.

Fig. 3 A section through the head of a Fundulus embryo eighteen days old. The specimen is shown in text-figure 1, no eyes are present, yet two perfectly differentiated crystalline lenses are seen in the sides of the head. A close examination of the photograph will reveal mesodermal cells between the lens and the brain.

Fig. 4. A section through one defective eye which possesses a lens of disproportionate size, while on the other side of the head two free lenses are seen, the lower small one protruding from the head. A second defective eye with a lens is found in a more posterior region of the head. Text-figure 4 represents this embryo as it appeared in life.

Fig. 5 A section through one of the very defective optic vesicles shown in fig. 2. The photograph illustrates the extreme lack of proportion which may exist between the size of the optic vesicle and its associated lens. It indicates also how small an amount of optic tissue may stimulate lens formation from the ectoderm.

1



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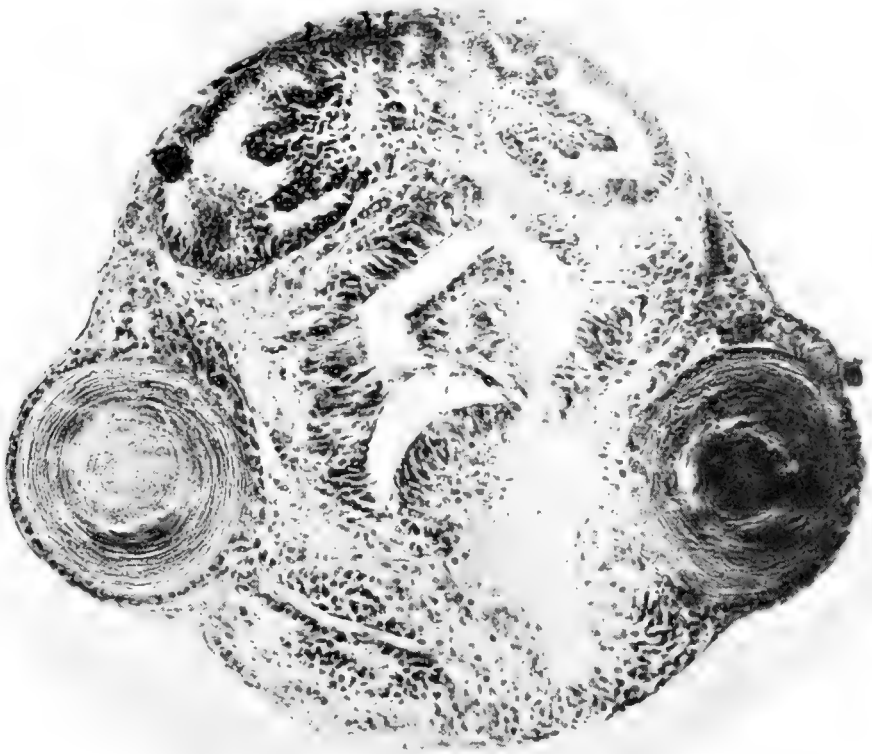

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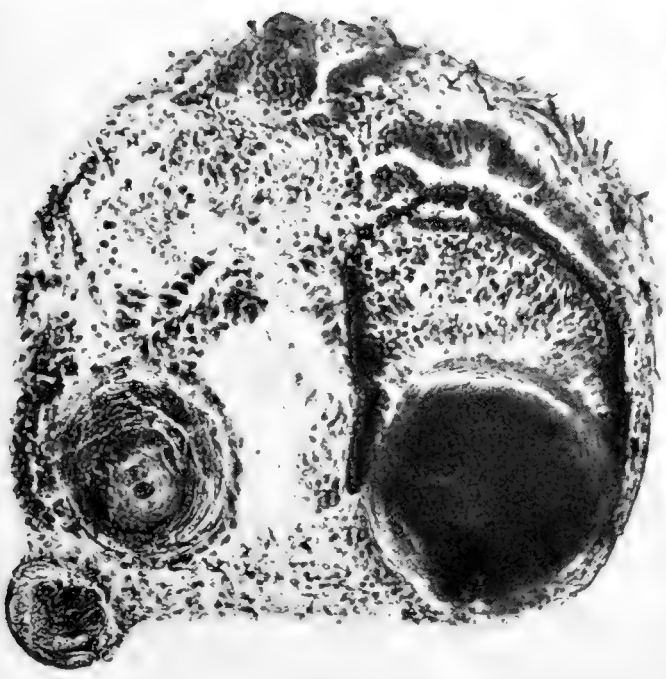

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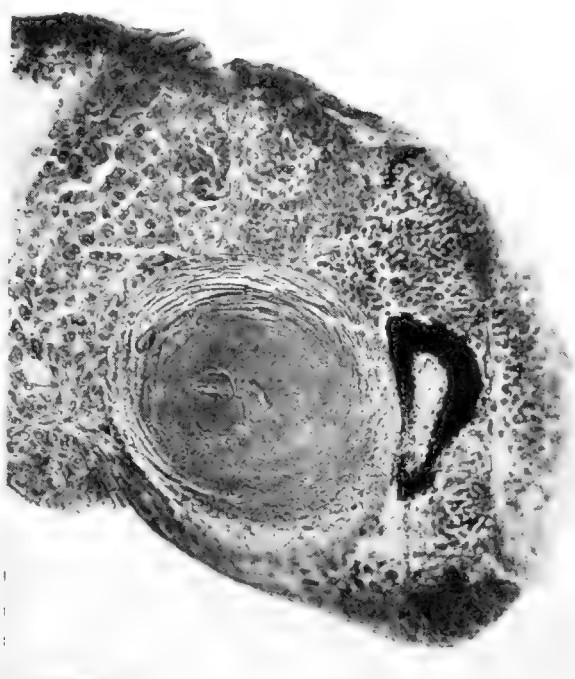

5

THE DEVELOPMENT OF ISOLATED BLASTOMERES OF THE FROG'S EGG

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WITH TWO FIGURES

After the numerous proofs furnished by various investigators, there is no question that differentiation begins very early in development, but the determination of the exact stage at which various differentiations begin, has been hampered by technical difficulties. The importance of overcoming these difficulties lies in the fact that it is only by an exact study of the early development that we can ever hope to know the mechanics of differentiation. The later stages are so complex that it is doubtful that they could be analysed even though the early stages were understood.

The frog's egg, owing to its large size, has been a favorable object of study. O. Hertwig,[1] after pricking one blastomere, found that a complete embryo was formed.

Roux,[2] in similar experiments obtained a half embryo from the uninjured blastomere, and explains Hertwig's observation by the fact that some of these half embryos became complete larvæ by a process of regulation which he called post-generation. This might occur in the following different ways: first, the half embryo might fold together on the side corresponding to the median plane and be transformed into a whole embryo of half size; second, the injured blastomere might recover from the operation and develop into the missing half; and third, the injured blastomere might be

[1]Arch. f. Mikroscopische Anat., 1893, XLII, p. 662.

[2]Ges. Abh. zur Entwicklungsmechanik der organismen, Leipzig, 1895.

reorganized under the influence of the half embryo into the missing half. The occurrence of post-generation, and especially the third type, has been denied by a number of investigators who repeated this experiment. Laquer[3] has reinvestigated the question.

This production of a half embryo from one blastomere is not due to the inability of the egg to produce more than one embryo, since Schultze[4] produced double forms by inverting the egg in the two-cell stage, and Morgan[5] produced a complete embryo from one blastomere by pricking the other blastomere and then inverting the egg. In order to determine whether this inversion of the egg and consequent stirring up of its contents is necessary for the production of a complete embryo from one blastomere, I attempted to find a frog's egg that would permit the complete removal of one blastomere without death of the egg. No one has hitherto been able to do this, although Roux[6] observed a partial but very abnormal development of extra-ovates of the frog's egg.

Last spring at Columbia, Missouri, I located the breeding places of Rana pipiens and Chorophilus triseriatus. The unsegmented eggs of both species could be collected in the ponds and small streams, or in the frog cages in the laboratory, the eggs of the latter species being more easily obtained.

I tried various methods of removing one blastomere in the two-cell stage. It was possible to suck out one blastomere with a capillary pipette connected with a rubber tube, one end of which was held in the mouth, but it was very difficult to pierce the jelly with the pipette without killing the egg, and the suction often removed both blastomeres. A better way of making a hole in the jelly was the time honored method of piercing it with a hot needle. A very coarse needle was used and heated to a temperature that would coagulate a portion of the blastomere into which it was thrust. On withdrawing the needle, the coagulated parts of the jelly and blastomere were pulled out, thus leaving a large hole

[3]Arch. f. Entwicklungs. 1909, XXVIII, p. 327.
[4]Arch. f. Entwicklungsmech., I. p. 298.
[5]Anat. Anz., 1895, X. p. 523.
[6]Jahresbericht d. Schles. Ges. f. vaterl. Cultur, Juni, 1887.

through which the remaining part of the blastomere could be extracted. The eggs of Chorophilus were more easily handled, since the jelly was softer and the egg itself did not collapse readily. This egg is not so small as to make operations difficult with the low power of the binocular microscope.

When the first cleavage furrow had extended almost around the egg, the greater part of the jelly was carefully removed with filter paper and the puncture made. The egg was then allowed to rest until the first cleavage plane had completely divided it. At this time the remains of the punctured blastomere were removed with the capillary pipette. In case the last remnants could not be removed, the egg was allowed to rest again until the remnants of the punctured blastomere turned pale, which was an indication that they were more brittle and could now be removed with a fine needle.

When the egg was punctured, the perivitelline space was obliterated by the escape of the contained fluid and the jelly pressed close to the egg, an opening was also made through which bacteria might enter; therefore, the eggs laid in the laboratory under a more sterile condition, gave better results than those laid in the ponds. Care was taken to keep the egg normally orientated in reference to the direction of gravity both during and after the operation.

The operated eggs were observed with the binocular microscope and the early cleavage noted. The gastrulation was watched through the sides and bottom of the glass dishes in which the eggs were kept. After the embryos became ciliated, some of them turned in a normal manner so that they could be observed in all aspects, from above.

A large number of eggs of both Rana pipiens and Chorophilus triseriatus were operated on. All of the former died, but of the latter a considerable number gastrulated and several reached the tadpole stage. Two were four days, three were five days and one was eight days old when preserved for sectioning.

The control eggs hatched in less than eight days, but the operated eggs were retarded in development and the resulting tadpoles were too weak to break through the jelly, although the twitching of their tails showed that the muscles were functional.

The development of the operated eggs was very similar to that of the normal, and quite different from that of the uninjured blastomeres in Roux's experiment. The remaining blastomere rounded up gradually after its partner had been removed, and the blastula, gastrula and later stages were *wholes* of half size. As the mortality was great, it is to be expected that defects would be observed in many of the specimens, but these defective specimens could not be interpreted as half embryos. The only defect that I could observe in the oldest specimen was the small size of the suckers,

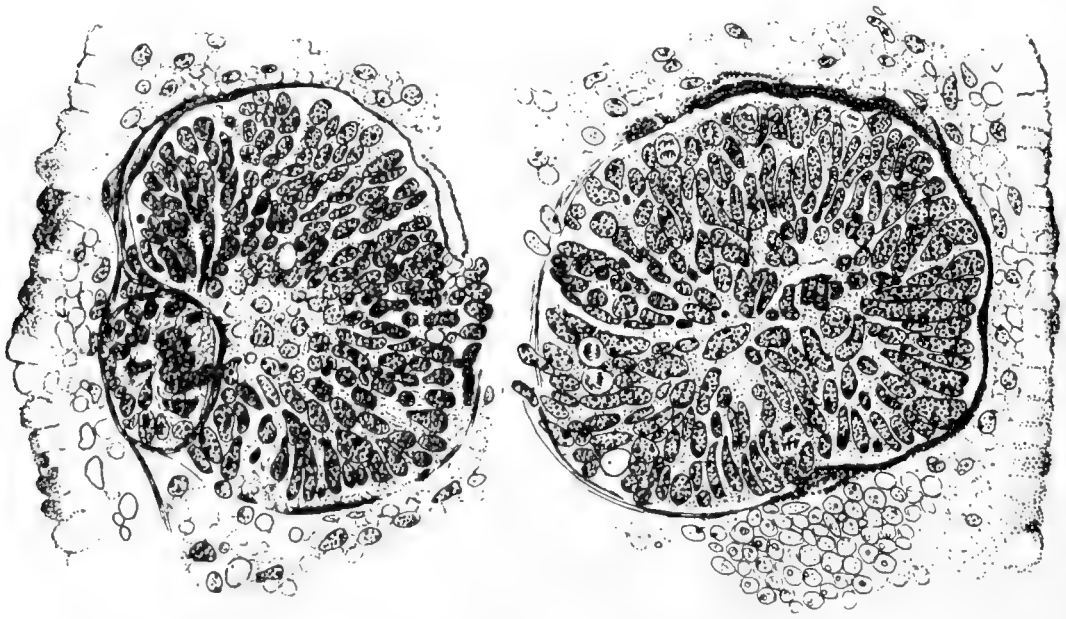

Fig. 1 Fig. 2

Fig. 1. Chorophilus triseriatus: a section of the left eye of a tadpole developed from one of the first two blastomeres.

Fig. 2. A section of the right eye of the same tadpole as in fig. 1.

before the pericardium began to swell so that the heart beats could no longer be observed.

Sections confirmed the observations on the living material and showed that the embryos were complete and not half-forms.

Figs. 1 and 2 represent the right and left eyes seen in transverse sections of the eight day larva. The right eye lacks a lens, and the left eye is not quite normal, yet these defects make no approach to those of a half-larva since they are not very extensive

and are not all on the same side. There is some displaced pigment in the center of the lens, and the cells of the optic cup have not preserved their normal arrangement. An examination of the ectoderm showed more or less disintegration, and the defects observed may be explained by the fact that the specimen was dying when it was taken out of the water to be preserved.

These differences in the two eyes of the eighty-day embryo from one blastomere, are the most striking differences between the right and left sides. The ear vesicles are almost identical on the two sides, and the same is true of the other paired organs. Furthermore, no general defect of the anterior, posterior, dorsal or ventral regions of the body could be found. The study of the five other embryos developed from operated eggs, and cut in serial sections also revealed complete forms.

Roux described right and left "hemiembryones laterales" and also "hemiembryones anteriores" developed from one blastomere in the experiments mentioned above.

Since the anterior end of the neural folds lies on one side of the egg between the equator and the animal pole, i. e., about the region in which the gray crescent appeared, and the dorsal side of the embryo is formed chiefly on this side, it is probable that in case the first cleavage plane were transverse to the plane of symmetry, it would divide the egg into the halves that would form the dorsal and ventral parts of the embryo respectively. Therefore the "hemiembryones anteriores" might better be described as "dorsales" as indicated by Przibram. Roux obtained no "posteriores" ("ventrales" of Przibram) and since a number of the eggs died, it is probable that those blastomeres which correspond to the ventral part of the embryo, and therefore contain no part of the gray crescent, are not so well adapted to independent development. In my experiments the position of the grey crescent as marking the future dorso-anterior region of the embryo was not recorded, but it seems probable from the results of Roux's experiment, that those isolated blastomeres containing no part of the grey crescent did not develop.

As noted above, Roux found that half-embryos sometimes became complete embryos by post-generation.

None of the three types of post-generation occurred in my experiments. The isolated blastomeres rounded up in a manner that might be compared to the first type of post-generation, but this regulation occurred before an embryo was formed, and might be distinguished as "immediate."

It appears then that the egg at the time of the first cleavage is differentiated in the direction of the primary axis, and furthermore in the direction of a plane passing through this axis and bisecting the grey crescent, so that the egg may be divided in this plane into two totipotent halves. The halves of the egg divided in any other plane may or may not be totipotent. The formation of a half embryo instead of a whole embryo from one blastomere of the two-cell stage is due to the presence and mechanical interference of the other blastomere.

In this connection may be mentioned the results of Endres, Herlitzka and Spemann on the development of the first two blastomeres of urodeles when separated by constriction with a fine hair. If the constriction was slight, the hair merely marked the plane of the first cleavage, if it was deeper, double monsters occurred, and if it was complete, two perfect embryos resulted.

In from 66 per cent to 75 per cent of the eggs, the first cleavage plane became a frontal plane of the embryo, and in the remainder, it became the median plane. In the latter case complete constriction gave rise to two complete embryos, but in the former case, complete constriction resulted in one complete embryo developed from the prospectively dorsal half of the egg, whereas the prospectively ventral half did not develop beyond gastrulation. Thus it appears that the eggs of the urodeles possess the same organization as the eggs of the anura, with the difference that the first cleavage plane is usually frontal in the former, and median in the latter in relation to the embryonic axes.

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NOTES ON THE MYOLOGY OF ANTHROPOPITHECUS NIGER AND PAPIO-THOTH IBEANUS

E. C. MacDOWELL

WITH FIVE FIGURES

Through the kindness of Dr. Spencer Trotter, professor of biology at Swarthmore College, I have been afforded the opportunity of dissecting a female chimpanzee, Anthropopithecus niger, which had just lost her milk dentition, and an adult male baboon, Papio-thoth ibeanus. The notes from these dissections were used by Dr. Trotter in May, 1908, as the basis of a communication to the Academy of Natural Sciences of Philadelphia.

It is my plan to describe certain muscles in these two forms, which are found to differ from the conditions normal in man, and to compare these with the results of previous investigators.

I wish to express here my appreciation of the many suggestions and the valuable assistance rendered by Dr. Trotter in the preparation of this paper.

MUSCULATURE OF THE UPPER LIMB

A. Muscles of the Shoulder

The *Pectoralis Major* in the chimpanzee, is divided into three parts which are not absolutely differentiated: (*a*) *pars abdominalis*; (*b*) *pars sternalis*; and (*c*) *pars clavicularis*. The first (*a*) arises from an aponeurosis with the rectus abdominus at the level of the seventh rib and from the sternum as far up as the third rib. It is inserted into the humerus, 3 cm. distal to its head, by a thin tendon, composed of loose fibers. This is covered by the more proximal border of the insertion of the pars costo-sternalis. The second part (*b*) is the most highly developed division of the group. It arises from the sternum and the sternal ends of the costal cartilages of the first, second, and third ribs. Its fibers are inserted

directly into the lip of the bicipital groove of the humerus, from the tendon of the pars abdominalis to the insertion of the deltoideus. The third division (*c*) which is, nearly as well developed as *b*, arises from the mesial two-thirds of the clavicle. It is inserted into the humerus for 5 cm. below its head, and overlaps the insertion of the second division (*b*). But slight differentiation is found between the main masses of the second and third divisions. There is an indication of a separation of a bundle of fibers anteriorly, which may represent a pars sternalis, but this bundle has no distinct insertion.

In the baboon, the pars clavicularis, is very poorly developed. The pars abdominalis (fig. 1, *A*) is a distinct ribbon-like band, which arises from an aponeurosis with the external oblique 5 cm. from the sternum, and extends from the seventh to the ninth rib. In the axilla, it is entirely covered by other muscles. It is inserted without a tendon, into the humerus close to its head. The pars costo-sternalis (fig. 1, *P*) is highly developed. It arises and is inserted as in man. The pars sternalis is very closely intermingled both with the deltoideus and the main portion of the pectoralis major. It arises from the sternum (a few fibers are supplied from the proximal end of the clavicle) and is inserted into the humerus, proximal to the insertion of the pars costo-sternalis.

This condition bears out Bischoff's statement, quoted by Primrose, that the pars clavicularis is wanting in the lower apes. Huntington and Michaëlis do not find it in Cynocephalus. Primrose considers that the attachment of the great pectoral has slowly crept out from the purely sternal origin, shown in the lower apes, to the origin which extends nearly two-thirds the way along the clavicle and joins the deltoideus as it does in my chimpanzee. Champneys found that the pectoralis major in his chimpanzee arose from eight ribs. The three divisions were not apparent, although two slips were differentiated. These arose from the fourth and fifth ribs respectively, and were fused with the main part of the muscle at the level of the lower border of the axilla. He describes the clavicular origin in the baboon as extending one-eighth of the way from the sternal end of the clavicle. Vrolik describes this muscle in his chimpanzee as having a clavicular

and a sternal part. In an orang, Duvernoy describes two parts, the *sterno-clavio-humerale* and the *sterno-humerale*, where as in a chimpanzee he found no divisions. Beddard found no connection with the clavicle in his orang. Owen describes three muscles in place of all the pectorals, namely the sterno-humeralis, costo-humeralis, and sterno-costo-humeralis.

The *Pectoralis minor* arises in the chimpanzee along a line extending from the first to the fourth ribs, 2 cm. from the sternum at its anterior, and 5 cm. at its posterior end. The muscle is divided into two separate digitations; the posterior digitation is subdivided into three. All the fibers quickly converge to the strong, round tendon, which is half as long as the muscular part. This is inserted into the capsule of the shoulder joint.

In my baboon and in a small Macacus monkey, the pectoralis minor is more developed. Distally it extends as far as the pectoralis major does in the first case, and in the second, from the second rib to the sixth. In both forms, it arises from the sternum itself and from the sternal ends of the costal cartilages; whereas, in man, it arises from the ends of the ribs themselves, thus forming a much less powerful muscle.

Champneys and Hepburn describe this muscle in chimpanzees as arising from the upper three ribs, and being inserted into the shoulder joint. Duvernoy did not find it divided into distinct parts. In the orang, Beddard found two parts, one from the third and fourth ribs, the other from the fourth, fifth and sixth. Hartmann describes the condition in the chimpanzee very much as I found it. His orang corresponded with Beddard's description. Primrose found the tendon of this muscle, in his orang, extending to the coracoid process, whence two ligments were traced to the head of the clavicle and to the acromion process, respectively. This was a new condition. Bardeen considers the insertion into the head of the humerus within the capsule, normal in primates below man. In a gorilla, Douvernoy describes this muscle (called by him "costo-caracoidien") as having two parts, one with six digitations inserted into the coracoid process, the other with two digitations joined to the short portion of the biceps after its insertion into the coracoid process.

The *Chondro-epitrochlearis* is found below the pars costo-sternalis in the chimpanzee. It arises between the second and fourth ribs from the fascia underlying the pars costo-sternalis, 2 cm. from its origin. It is composed of three strands lying parallel to those of the superficial muscle. It is inserted into the humerus by a thin strap-shaped tendon, which adheres closely to the tendon of the upper sheet at its insertion into the humerus.

Bardeen describes the muscle when found in man as follows:

> This is a slip which springs . . . . from the thoraco-abdominal fascia beneath the pectoralis major . . . . and extends on the inner arm to the intertubercular (bicipital) groove . . . . It is found in 12 to 20 per cent of human bodies (Le Double) aud occurs normally in many of the lower mammals.

Duckworth describes in a gorilla the separation of the posterior fibers of the pectoralis major to form a separate muscle, the "pectoralis abdominalis," and identifies it with the chondro-epitrochlearis. However, it seems clear that the pars abdominalis in my chimpanzee must correspond to his "pectoralis abdominalis." So, if this slip under question corresponds to any muscle in man, it must correspond to the chondro-epitrochlearis. This seems to indicate that the abdominal portion of the pectoralis major and the chondro-epitrochlearis should not be identified. In man, this muscle has been found in one out of eight (Le Double).

In the baboon, I find a muscle that arises from the rectus abdominis and the fascia and cartilage of the seventh rib, below the origin of the abdominal portion of the pectoralis major. Its fibers form a strip 5 cm. wide, which runs parallel to the sternum. At the level of the second rib, it becomes cartilaginous, and is inserted into the costal cartilage and the surrounding fascia. It seems probable that this muscle should be considered a portion of the rectus abdominis that has been stranded in the thoracic region upon the retreat of this muscle.

Wiedersheim states that the abdominis in Amphibians extends from the tail to the head, and that this muscle crept back with the development of the pectorals, which needed a solid frame for their insertions. In the lower primates, this muscle regularly

reaches well up into the thoracic region, while in man it has occasionally been found to extend as far up as the second rib. Bardeen says that a more primitive condition, in which the muscle extended to the neck, is frequently indicated in man by aponeurotic slips or muscle slips on the upper part of the thorax. In my Macacus, I find the rectus abdominis extending up to the fifth rib.

The *Subclavius* is found in the chimpanzee to arise from the first rib 3.5 cm. from the sternum, by a short tendon. The belly is .75 cm. in diameter. Its fibers are inserted into the distal half of the clavicle. Some of these fibers are closely related to the conoid ligament. In the Macacus, this muscle is applied to the clavicle for most of its length.

Vrolik found this as in man; Beddard describes it as inserted on the proximal half of the clavicle and ensheathed by the coraco-clavicular ligament. In his orang, Hepburn found an additional slip from the second rib, while in the orang of Primrose it was in no way continuous with the coraco-clavicular ligament, and was poorly developed.

In the chimpanzee, I also find a *Sterno-chondro-scapularis* arising as a tendon inserted into the first rib, and affixed to the coracoid process internally to the attachment of the short head of the biceps. The only muscle fibers that occur are in a small group at the coracoid attachment, and in a still smaller one at the costal insertion. Champneys describes such a fibrous band and notes a partial fusion with the subclavius at the anterior end. He quotes Rolleston as calling this the representative of the long coracoid of birds. Le Double reports it as occurring in man in two out of forty cases.

The *Deltoideus* presents only slight irregularities. In the chimpanzee and in the baboon, the connection between its most ventral border and the clavicular portion of the pectoralis major is unusually close. In the baboon, the short attachment of the pectoralis to the clavicle gives the deltoideus a remarkable long clavicular origin.

Champneys and Michaëlis say that it arose from nearly all the clavicle in their baboons, while Hepburn states that the close joining of the deltoideus with the clavicular portion of the pec-

toralis is common in all anthropoid apes. Beddard describes this muscle in Anthropopithecus calvus as being relatively as large as in man, and Hartmann says that it is well developed in anthropoids generally.

B. Muscles of the Arm

The *Latissimus dorsi* arises in my chimpanzee from the four posterior lumbar vertebrae closely mingled with the posterior border of the trapezius, from the crest of the ilium, and by interdigitations with the obliquus externus abdominis from the four posterior ribs. The muscle is inserted into the bicipital groove by a strong flat tendon 2 cm. wide and 4 cm. long, superficial to the proximal half of the broad insertion of the teres major. There is no connecting fasciculus between these two muscles. The fibers of the *Latissimo-condyloideus* (dorsi-epitrochlearis) arise from the flat tendon of the latissimus dorsi at its distal end, and continue down the arm in a belly 1.3 cm. in diameter to be firmly inserted by a strong flat tendon 4 cm. long into the internal condyle of the humerus. This muscle receives a fasciculus from the coracobrachialis.

In the baboon, the tendon of the latissimus dorsi (fig. 1, *E*) is entirely distal to that of the teres major (fig. 1, *R*). A large fasciculus (fig. 1, *V*) is given off from the superior border of the latissimus dorsi to be inserted into the lower border of the broad flat tendon of the teres major before the insertion of the former into the humerus. The latissimo-condyloideus (fig. 1, *U*) is large and well developed. It arises mainly from the muscle fibers of the latissimus dorsi. Indeed, many of the fibers of the latissimus dorsi seem to be continuous with those of this muscle. It is inserted into the long head of the triceps and the brachial fascia just above the elbow. In this way, the insertion of the latissimus dorsi is practically extended to the olecranon process, and a very long and powerful muscle is formed, which may be called the *Latissimo-dorsi-condyloideus*. In the Macacus, the latissimo condyloideus is very poorly developed. Its fibers are inserted directly into the fascia over the triceps.

In his orang, Primrose found a fasciculus from the latissimus dorsi to the tendon of the teres major, similar to the one I have described in the baboon. Hepburn describes a similar condition in a chimpanzee and in an orang. He states that the tendons of the latissimus dorsi and of the teres major are joined in the gibbon. The fascicular connection in the higher forms denotes an earlier joining of the teres muscles; however, in my baboon,

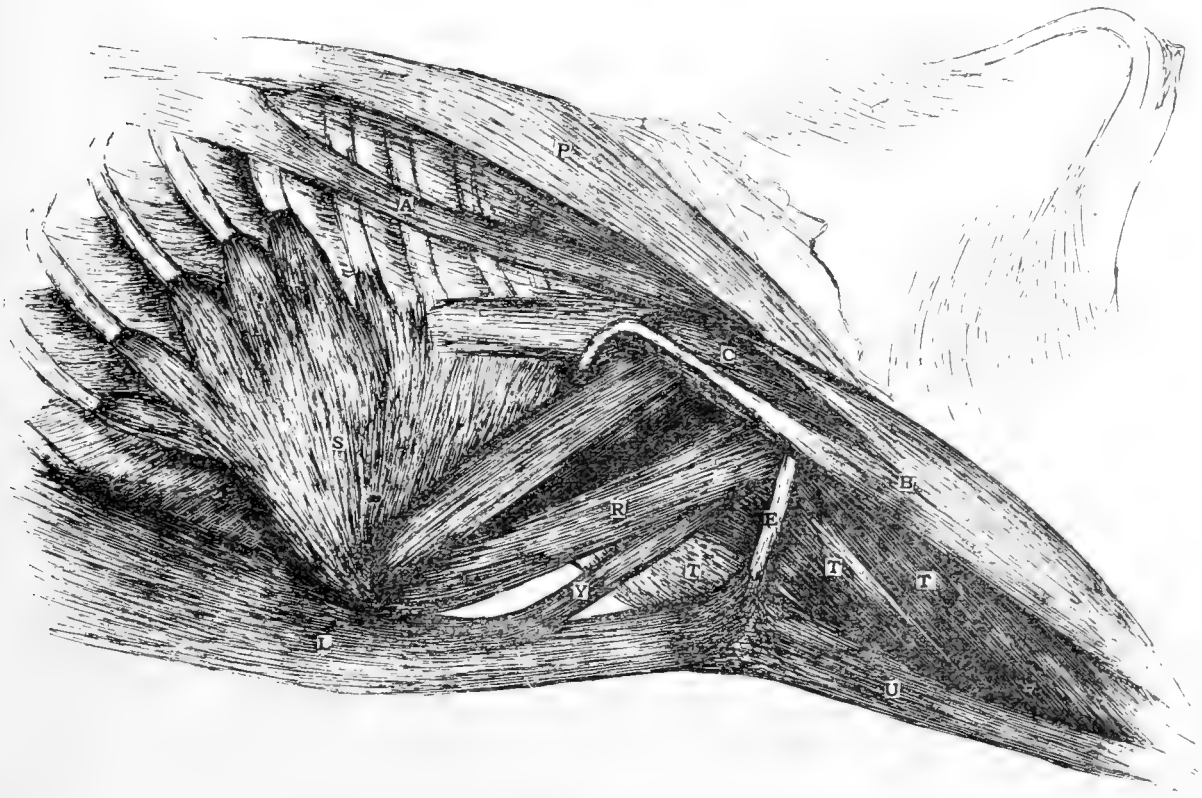

Fig. 1. The side view of the baboon; the left arm is raised to show the insertion. of the Latissimus dorsi. *A*, pars abdominalis of pectoralis major; *B*, biceps; *C*, Coracobrachialis; *E*, Tendon of insertion of Latissimusdorsi; *L*, Lattissimus dorsi; *P*, Pars costo-sternalis of Pectoralis major; *R*. Teres Major; *S*, Serratus anterior; *T*, Triceps; *U*, Lattissimo-condyloideus; *Y*, Fasciculus from Latissimus dorsi to teres major.

where this is present, the insertions themselves are further separated than in my chimpanzee, where it is absent. Vrolik, Chapman, Hepburn, Beddard, Duvernoy and so many others describe the latissimo condyloideus that we may well believe that it is present in all anthropoids. It is interesting to note that it is poorly developed in the chimpanzee, for in man it occurs only as

an irregularity. Gray describes such a muscle in man, and says that Dr. Struthers found it in 7.6 per cent of bodies. Macalister, Le Double, Wood and Bardeen all found this in about 5 per cent of cases. Patterson reports its occurrence as "occasional." Bardeen says it is normally represented by a fascial slip. Hartmann describes two insertions of this muscle in the chimpanzee, one into the condyle, the other into the middle or inner head of the triceps. He quotes Bischoff as describing in the chimpanzee an insertion into the fascia over the biceps; in the baboon, an insertion into the intermuscular septum and the internal condyle. Michaëlis found it inserted into the triceps in his orang. In the gorilla, Hepburn describes this muscle as arising from the coracoid process.

In the chimpanzee, the *Coracobrachialis* has two divisions. Their common origin is from the tip of the coracoid process, and from the tendon of the short head of the biceps, with which it is intimately related. The first division is inserted into the humerus between the insertions of the pectorals and the latissimus dorsi. This is accompanied by a strong tendon and tendinous bands, which pass through the body of the muscle. At a point 5 cm. from the origin, the main division becomes separated from the belly of the biceps. This division also has tendinous bands from which several of the fibers of the biceps take their origin before the bellies separate. The insertion of this division of the coracobrachialis extends from the distal limit of the insertion of the first division to a point five-sixths of the way down the humerus. Distally, it joins the medial head of the biceps by an aponeurosis.

In the baboon (fig. 1, *C.*) the proximal division is highly developed. It adheres closely to the humerus as a broad flat belly. In the Macacus, also, this secondary head is well developed. It arises as in the other forms and immediately leaves the common tendon to be attached by a fleshy insertion close up under the head of the humerus, its fibers being perpendicular to the long axis of the humerus. There is complete separation of the parts. The lower belly extends straight down to be inserted into the lower third of the humerus.

From the comparison of all mammalian forms, Wood concludes

that this muscle is typically composed of three parts, all of which arise from the coracoid process. The upper head is regularly lacking in man, and this seems to be the case in my chimpanzee. However, it is present in the baboon and in the Macacus. Champneys, Hepburn, and Beddard describes two divisions in the chimpanzee, while Duvernoy and Vrolick describes one. Hepburn found elements of all three divisions in his gorilla. Lubosch found two divisions in a human body (see table I).

TABLE I

ANIMAL	REPORTED BY	CORACOBRACHIALIS
Chimpanzee	Hepburn	2 divisions
Chimpanzee	Champneys	2 divisions
Chimpanzee	Beddard	2 divisions
Chimpanzee	Duvernoy	1 division
Chimpanzee	Vrolick	1 division
Chimpanzee	Author	1 division
Baboon	Author	2 divisions
Macacus	Author	2 divisions
Gorilla	Hepburn	elements of 3 divisions
Orang	Primrose	2 divisions
Typical Mammal	Wood	3 divisions
Man	Lubosch	2 divisions as a variation

The *Triceps Brachii* has but little variation from the muscle in man. In the chimpanzee, the three heads are intermingled a short distance from their origins. The long head arises from the axillary border of the scapula for nearly its whole length, and distally from the deltoideus and the surrounding fascia. This origin is semi-cartilaginous. Its belly is flat and wide before its incorporation with the other heads. It lies between the teres major and the deltoideus. The long head in the baboon arises by a tendon closely associated with the muscle fibers from the axillary border of the scapula between the teres major and teres minor and is lost immediately in the main belly of the triceps; thus it has a triangular shape. A flat slip of muscle 1.5 cm. wide arises from the axillary border of the scapula just beyond the distal limit of the origin of the long head. This slip extends half way down the arm parallel to the long head of the triceps and is inserted into the fascia cover-

ing the main belly of the triceps, just proximal to the insertion of the lattissimo condyloideus. The whole muscle is characterized by the close interlacing of the parts. The Macacus shows a separation of three parts half way down the arm.

Hepburn found this muscle strongly developed in all anthropoids, and Beddard describes it as in man. Bardeen says a fourth head is frequently found in man as a variation. Le Double reported 20 such cases.

C. Muscles of the Forearm

The *Palmaris longus* arises in the chimpanzee from the common tendon from the internal condyle external to the flexor carpi radialis, and is closely joined to it. It becomes separate 8 cm. from its origin and is inserted into a slender tendon which is inserted into the proximal, radial aspect of the carpal ligament and the neighboring palmar fascia.

Beddard, Vrolik, Champneys, and Rolleston (quoted by Champneys) found the same arrangement in the chimpanzee. In the orang, Duvernoy and Michaëlis describe it as being very strong. Primrose found a slip to the thumb from which arose a part of the abductor brevis. It is wanting in the gorilla (Hartmann, Hepburn, and Duvernoy). Forster found it well developed in his "Papua neugeborenen." Bardeen says that it was once a third flexor to the digits (flexor longus superficialis). McMurrich states that this muscle seems to have been the first to separate from the common flexor mass. Adachi reports it lacking in 20.4 per cent of European men but only in 3.9 per cent of Japanese. Le Double did not find it in 11.2 per cent of bodies.

The *Flexor digitorum sublimis* (perforatus) is characterized in the chimpanzee by the extreme separation of the bellies going to the different digits. Although closely bound together by fascia, the bellies can be traced to definite and distinct origins.

A. The division for the index finger arises from the coronoid process of the ulna and from the tendinous sheath of the flexor carpi radialis by two groups of loosely bound fibers, which become at once incorporated into the main belly. The fibers are inserted

in a penniform manner into a tendon which extends nearly the whole length of the muscle. One third of the way from its origin to its insertion, the tendon lies on its radial aspect, the fibers being inserted upon the ulnar side of the tendon. Beyond this, other fibers arise from the radial side of the tendon, and those on the ulnar side twist around so that the tendon is on the ulnar side of the belly. This gives the muscle a digastric appearance. The tendon is inserted into the base of the second phalanx, as is the case in man. All four bellies are inserted into their respective digits in the same manner. Two fasciculi are given off at the point where this belly is twisted, to join the belly for the third digit.

B. The division for the third digit arises (*a*) from the tendinous sheath of the flexor carpi radialis, superficially to the origin of the first head, by three separate divisions, each 1 cm. wide, at points 2, 4. and 5 cm. respectively from the insertion of the flexor carpi radialis into the internal condyle of the humerus; (*b*) along the oblique line of the radius from a point 4 cm. from the distal end of the bone to a point 6 cm. from its proximal end, and from the brachial fascia neighboring this line. The bundles of the proximal origin continue down the arm loosely joined. The radial head joins this tendon obliquely on its radial border. This whole belly is superficial to the first one, and lies on its radial side.

C. The division for the fourth digit arises (1) most superficially from the tendinous sheath of the flexor carpi radialis by three strap-like divisions (*a*) 5 cm. distal to the external condyle, (*b*) more distal, 1 cm. broad, (*c*) distal to (*b*) 2 cm. broad; *a* and *b* unite but *c* does not join the other two for two thirds of the way down the arm: and (2) from the intermuscular septum between it and the flexor carpi ulnaris, near its insertion into the internal condyle. All these fibers are inserted in a penniform manner upon the inner surface of the tendon, which extends half way up the arm.

D. The division for the fifth digit arises from the intermuscular septum between it and the flexor carpi ulnaris, at a point 6 cm. from its origin. This division is very small and is entirely covered by the flexor-carpi ulnaris. The tendon is long and slender. From this it will be seen that the independence of the origins is well marked, and, with one exception, there is not the slightest connection between any of the main divisions.

In the right hand, there is a peculiar abnormality in the insertion of the tendon to the middle finger. It is firmly affixed to the base of the proximal phalanx. The tendon of the flexor profundus is also inserted at this point, where the fibers of the two tendons are closely interlaced. From here to the base of the second phalanx extends a strong tendon, which is so short as not to allow the first and second phalanges to form an angle greater than 95°. A second tendon internal to this, extends from the base of the first phalanx to the terminal phalanx. This tendon is long enough to give the terminal phalanx free movement and, as there are a few muscle fibers at its origin, it must have had a slight power to flex the digit. From their relative positions and insertions, it seems fair to suppose that these tendons correspond to the normal tendons of the flexor digitorum sublimis and profundus.

In the baboon, the opposite extreme is found. The sublimis consists of but one belly (fig. 2, *S*), which takes its origin from the common tendon attached to the medial epicondyle of the humerus. The radial origin is not represented even by a fasciculus. The belly is no longer than any of the other superficial flexors. The special tendons to the digits do not separate until the common tendon has passed the carpal ligament.

In the chimpanzee, Champneys found an additional origin from the radius only for the division to the second digit; Hepburn reports that the tendons for the second and fifth digits had radial origins, while those for the third and fourth digits had ulnar origins. Macalister, d'Alix and Gratiolet found no radial origin. Chudzinski (quoted by Le Double) found that in all races of men there was a digastric muscle in the deep part of this muscle.

TABLE II

ANIMAL	REPORTED BY	RADIAL ORIGIN OF FLEX. DIG. SUBLIMIS
Chimpanzee	Macalister	absent
Chimpanzee	Gratiolet-Alix	absent
Chimpanzee	Hepburn	present-supplies digits II–V
Chimpanzee	Champneys	present–supplies digits II
Chimpanzee	Author	present-supplies digits II, III, V
Baboon	Author	absent
Man	LeDouble	variable—may be absent

McMurrich ('03) shows that the sublimis has been differentiated from a common flexor mass similar to that found in Monotremes and, little by little, has developed until it includes all the condylar portions of the original muscle. In the chimpanzee, the further development of a radial origin does not seem to be constant. Even in man, this origin is variable and may be wanting. Complete separation of the divisions for the digits is also found (Le Double). (See table II.)

The *Flexor digitorum profundus* (perforans) arises in the chimpanzee (I) (*a*) from the oblique line of the radius internal to the origin of the second head of the sublimis, and (*b*) from the radial half of the interosseous membrane; it also arises (II) (*a*), from the dorsal surface of the ulna along its upper two-thirds by an aponeurotic septum between it and the flexor carpi ulnaris, (*b*) from the proximal half of the under surface of the ulna, and (*c*), from the ulnar half of the interosseous membrane. The radial head (*a*) supplies a large perforating tendon for the second digit; the fibers are inserted upon the radial border of this tendon. This belly corresponds in its origin to the flexor pollicis longus in man, although in my chimpanzee, no connection can be found between this division and the thumb. However, a fine tendon is found arising from the annular ligament and from the surrounding palmar fascia, which is inserted into the terminal phalanx. Such an insertion would correspond to that of the flexor pollicis longus in man. As there was no muscle connected with this tendon, it seems that there could have been no flection of the terminal digit. Le Double although never himself finding the flexor pollicis longus lacking in man, cites Gruber, Wagstaffe, and Fromont as having found a case each where this muscle was represented by a thin tendon from the sesamoid bone of the metacarpo-phalangeal articulation to the terminal phalanx. The tendons for the third and fourth digits extend far up into the fibers from the ulnar origin. They show a tendency to divide into threads and two bellies are closely associated all the way down. Just after leaving the annular ligament, the tendons are joined by a strong transverse sheet of tendon 3 cm. broad, from which arise two of the lumbricales. The division for the fifth digit also arises from the ulna as a small

independent head. Its tendon is divided all the way to the wrist. The irregular insertion of the tendon to the middle finger in the right hand has been previously described.

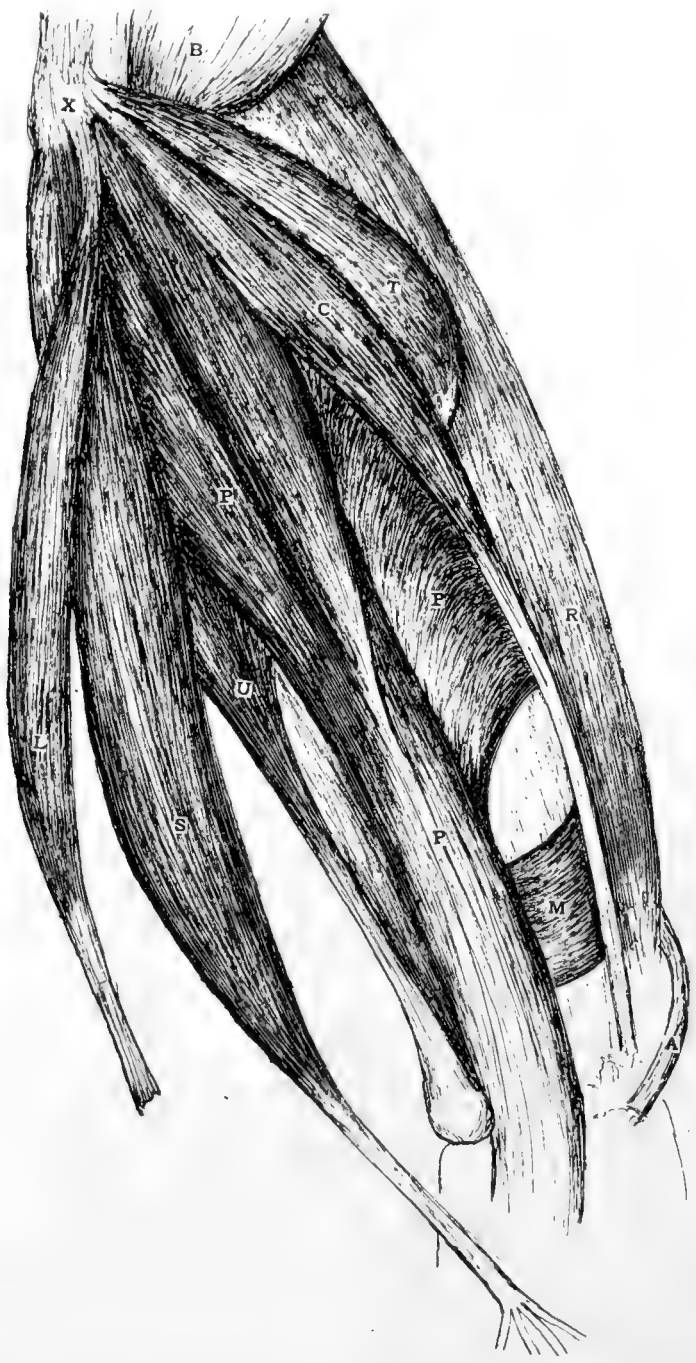

FIG. 2. Dissection of left forearm of the baboon, to show the arrangement of the flexor digitorum profundus. The tendon of the Palmaris longus has been cut, and the flexor digitorum sublimis has been pushed over to the right. *A*, Tendon of Abductor pollicis longus; *B*, Biceps; *C*, Flexor carpi radialis; *L*, Palmaris longus; *M*, Pronator quadratus; *P*, Flexor digitorum profundus; *S*, Flexor digitorum sublimis; *U*, Flexor carpi ulnaris; *X*, Internal condyle of humerus.

The flexor digitorum profundus in the baboon (fig. 2, *P*) shows great consolidation. It arises (*a*) from the oblique line of the radius as far as the pronator quadratus, (*b*) from the posterior border of the ulna, extending from the olecranon process to a point 8 cm. from the distal and, (*c*) from the common flexor tendon from the internal condyle of the humerus; the fibers from these various origins are joined in a broad flat tendon 2 cm. wide, which extends half way up the arm. It is heavily grooved, indicating the elements of the tendons for the digits, but these tendons are not differentiated until the tendon reaches the palm. The origin (*a*) from the radius corresponds to that of the flexor pollicis longus, which, as in the chimpanzee, is still undifferentiated from the profundus. However, a strong round tendon, supplying the pollex (fig. 3, *T*), is given off from the common tendon just after passing the ligament.

In a gibbon, Hepburn found an origin of the profundus from the internal condyle, which, he says, clearly shows the relation between the two flexors. The condylar segment has become the sublimis. The condition in my baboon shows the profundus, including fibers which in the higher forms are included in the sublimis. The separation of the flexors into individual bellies is considered, by Bardeen, to be associated with the refinement of the movements of the fingers. Champneys, Beddard, and Hepburn found no tendon to the pollex, but name the division to the index the flexor pollicis longus, and describe a small tendon given off from this to the pollex. Testut calls the separated divisions to the index in his chimpanzee a part of the profundus. This agrees with McMurrich's conclusions that the flexor pollicis longus should not be said to be missing in monkeys, nor be described as fused with the profundus, but rather as still undifferentiated from this muscle. In the chimpanzee, Keith found no tendon to the pollex in ten out of twenty-five cases; Vrolik describes a tendon from the flexor brevis digitorum that is inserted into the terminal phalanx, which seems to take the place of the tendon of the flexor pollicis longus. Bischoff found no tendon to the pollex in the gorilla. Henle, Wood, and Turner (quoted by Champneys) observed in man a muscular slip from the flexor pollicis longus which sent

a tendon to join that of the profundus for the index. Michaëlis describes three separate divisions of the profundus in his baboon. A tendon for the thumb was given off from the head that supplied the fourth digit. Champney's description of his baboon corresponds to mine. Testut claims that, with the exception of

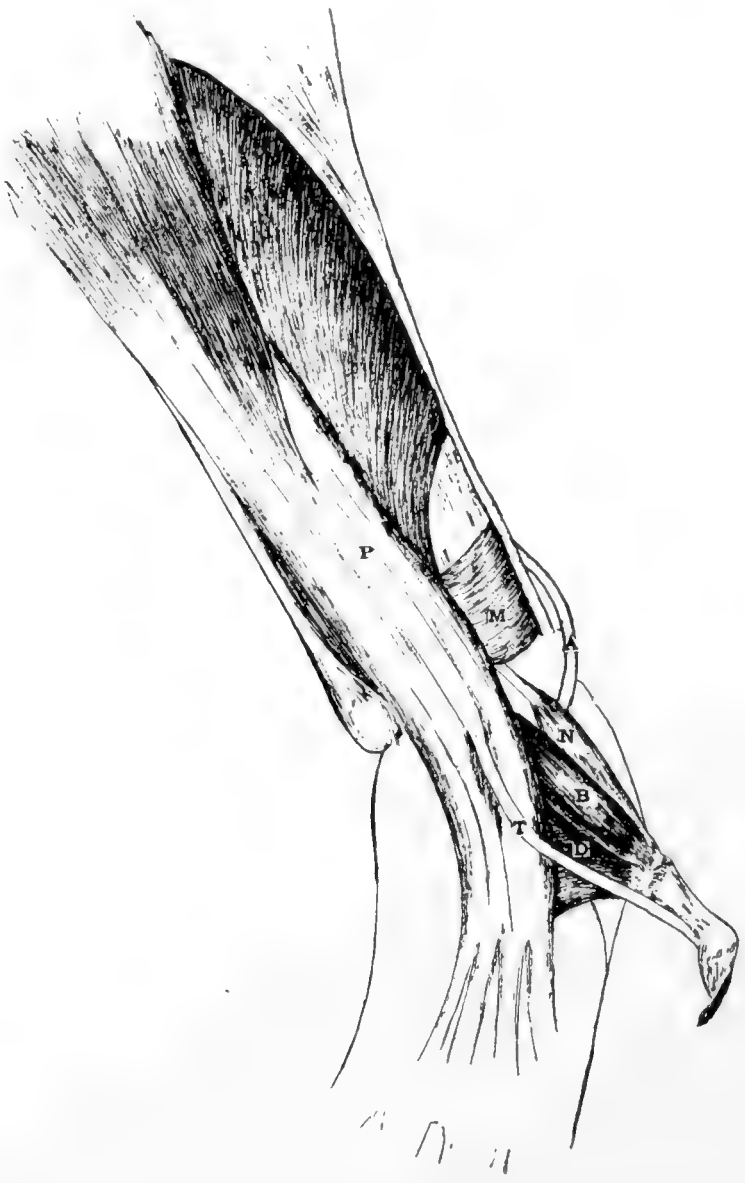

FIG. 3. Dissection of the wrist and muscles of the thumb of the baboon, to show the relations of the tendon of the flexor pollicis longus to the profundus. The superficial muscles have been removed. *A*, Tendon of Abductor pollicis longus; *B*, Abductor pollicis brevis; *D*, Abductor pollicis; *M*, Provator quadratus; *P*, Common tendon of flexor digitorum profundus; *T*, Tendon to pollex, corresponding to that of the flexor pollicis longus, in Man.

nycticebus tardigradus, there is no separate flexor pollicis longus in any of the monkeys. He believed that this is an important distinction between man and ape; however, he does not believe with Gratiolet and Alix that its separation in man and blending in apes is any hindrance to the evolution theory. For he had found in human examples all the main types of the coalescence of this muscle, as variations. Dally also answered Gratiolet by arguing that if man did not use it, this muscle would soon become undeveloped. He believed that the development of this muscle has come with civilization, and that it was far less developed in primitive man.

In the chimpanzee, the *Abductor pollicis longus* (extensor ossis metacarpi pollicis) arises from the interosseous membrane, and the intermuscular septa between it and the extensor carpi radialis brevis and the extensor indicis proprius. It gives off two tendons: one of these is inserted into the trapezium, the other into the base of the metacarpal bone of the thumb. No sesamoid bone is found in its tendon.

Beddard describes a similar division and insertion in his chimpanzee. In man, these same conditions are found at times, which closely connect the hand of man to that of the ape (Le Double). In a gorilla, Duvernoy describes these divisions as the cubito-sus-metacarpien and the cubito-sus-trapezien. Hartmann quotes Bischoff as saying that the long abductor in the orang and in the baboon is as in man, but that division is found in the gorilla, chimpanzee, and macacus. It cannot be said that one of these tendons belongs to the extensor pollicis brevis, for Hartmann, Duvernoy and Vrolik have found an extensor pollicis brevis coexisting with a "cubito-sus-metacarpien" which was inserted into the metacarpal of the thumb.

The *Extensor digiti quinti proprius* in the chimpanzee arises from the ulna below the origin of the extensor digitorum communis. The fibers are inserted into a strong, flat tendon that passes through a special compartment of the annular ligament, to be inserted into the fascia covering the second phalanx of the fifth digit. It seems correct to name this the special extensor of the fifth digit, since in its origin and in its passage through the ligament, as well as in its insertion, it corresponds to this muscle in man.

Beddard says that this muscle was lacking in his chimpanzee, or else the division of the extensor communis to the fifth digit was lacking (which seems more reasonable, since he describes a tendon to the fifth digit that passed through a special compartment in the ligament). As will be seen later, the division for the fifth digit of the communis is present in my chimpanzee, as well as this special extensor. Champneys found the fifth digit of the right hand of his chimpanzee supplied only by a slip from the tendon of the communis. The left hand was as in man. Vrolik describes the special extensor as the only tendon to the fifth digit, but Michaëlis claims that this is the ulnar belly of the extensor digitorum communis that has become entirely separated.

The *Extensor digitorum communis* in the chimpanzee is separated into four bellies half way down the arm. It arises by a strong tendinous sheath from the external condyle, from the intermuscular septa between it and the extensor carpi ulnaris, from the ulna, radius, and the interosseous septum. In the left hand, two divisions of this muscle send tendons to the fourth digit. The more radial and stronger arises from the condyle and is joined to the tendon of the third digit by a short tendinous fasciculus. The other division arises from the whole length of the ulna. A slender fasciculus arises from the ulnar border of this division 1.5 cm. from the annular ligament. It is provided with a thread-like tendon which divides 5 cm. from the ligament. The radial half re-divides close to the metacarpo-phalangeal articulation, and sends one branch to the tendon for the fourth digit, while the other rejoins the ulnar half of the tendon from the first division, which is inserted along with the extensor digiti quinti proprius. This slip represents the division of the communis for the fifth digit. In the right hand, there are three separate tendons for the fourth digit, besides the connection with the fasciculus for the fifth digit.

Vrolik and Macalister found no tendon for the fifth digit in their chimpanzees. Primrose describes a slip in his orang that corresponds to the one just described, but calls it the extensor digiti quinti proprius (extensor minimi digiti). In man, the tendon for the fifth digit is often absent or rudimentary.

The *Extensor indicis proprius* arises in the chimpanzee from the

distal four-fifths of the ulna. It is divided into three parts which give off three tendons. Two of these are large and are inserted into the extensor expansion over the dorsal aspect of the proximal phalanges of the second and third digits respectively, on the ulnar side. The third tendon is very small and is closely applied to the tendon for the second digit, from which it becomes separated 1.5 cm. before its insertion into the radial side of the extensor expansion of the index. Similar conditions are found in the baboon.

Beddard describes this muscle in a chimpanzee as having but one strong tendon and that joining the branch of the communis for that digit. Mayer found it small or entirely lacking in the chimpanzee and in the orang. In the chimpanzees of Hartmann and Macalister, an extra tendon for the third digit was found. Champneys found such a condition in his Cynocephalus and Le Double reports its occurrence in man. Duckworth describes the double extensors to all the fingers (formed by branches of the indicis proprius and digiti quinti proprius to the third and fourth digits respectively) as being constant in many apes. Again, Primrose quotes Huxley as stating that there were originally two sets of extensors, just as there were two sets of flexors, and that this is indicated by the dividing of the special extensors of the second and fifth digits. It may be remembered that I do not find a division of the tendon of the extensor digiti quinti proprius, but I do find a division in the tendon of the fasciculus of the communis for the fifth digit which could not correspond to a deep flexor, as it was joined directly to the tendon of the communis.

II. MUSCULATURE OF THE LOWER EXTREMITY

A. Muscles of the Hip

The *Scansorius* is present in the chimpanzee. It arises from the anterior border of the ilium below the anterior superior spine, and its insertion is into the anterior border of the great trochanter, together with the gluteus minimus. These two *muscles are independent* for two thirds of the way from their origins.

In his chimpanzee, Beddard found the scansorius well devel-

oped, but not separated from the gluteus minimus. Primrose found it in his orang, and quotes Hepburn, Huxley, and Owen as finding it separate in the chimpanzee. Bischoff and Champneys describe it closely connected to the gluteus minimus. In Forster's "Papua-neugeborenen" this muscle was well separated on one side. Bardeen says that a special fasciculus from the anterior margin of the gluteus minimus in men corresponds to the scansorius and is frequently called invertor femoris or small anterior gluteal. This muscle was discovered in man by Haughton (Le Double); in the chimpanzee by Troill and in the orang by Bischoff.

The *Gluteus maximus* in the chimpanzee arises (1) from the crest of the ilium by a broad tendinous sheet from the sacrum and coccyx and (2) from the tuberosity of the ischium, anterior to the insertions of the semi-membranous and biceps femoris. The fibers from the first origin converge towards the head of the femur, over which they pass to join a tendon, which is inserted upon the external aspect of the femur, half way down. The insertion of the second part, ischio-femoralis (Duvernoy), is into the tendon just described along the femur. Some of these fibers continue down the leg all the way to the external condyle, and are connected to the vastus externus by a septum.

Vrolick and Beddard describe the same structure in their chimpanzees. Duckworth reports an insertion largely into the posterior surface of the femur in the Lemuroidea. In the Cercopithecidea, the femoral insertion is very small. Hartmann found a tendon of insertion in Anthropoids descending far down towards the knee. I find a very narrow insertion in my macacus. Wiedersheim states that the special development of this muscle is peculiarly human.

B. Muscles of the Leg

In the chimpanzee, the *Plantaris* arises from the tendon of the lateral head of the gastrocnemius and from the lateral line of the bifurcation of the linea aspera, just proximal to the origin of the head of the gastrocnemius. The belly is slender, 6 cm. long and passes into a slender tendon that lies beneath the medial head of

the gastrocnemius to be inserted into the medial border of the Tendo Achillis. In comparison with man, this belly is longer. The tibia of the chimpanzee is 20 cm. long, that of man from 45 to 50 cm. (Gray). In both cases the plantaris is 6 cm. long.

Beddard found this muscle present in the chimpanzee, while Champneys did not. Michaëlis says it was well developed in the baboon but absent in the chimpanzee and orang. It was lacking in the orang of Primrose. Duckworth says it is never found in the gorilla, but frequently in the chimpanzee; it is normal in the Cercopithecidæ and Lemuroides. Hartmann found it in his chimpanzee, although he says Bischoff and Brühl at first denied its existence. He did not find it in either the gorilla, the orang, or the gibbon. Kohlburgge reports its absence in the chimpanzee in 43 per cent of cases and Loth in 45.7 per cent. Le Double found rather frequent cases of the absence of this muscle in man. However, in colored races its absence is rare. The plantaris, as well as the palmaris its homologue in the arm, exemplifies a degenerating muscle. Wiedersheim (p. 109) states that "as an original flexor (the plantaris) must have begun to degenerate from the time the plantar fascia became secondarily attached to the calcaneus, and helped in the formation of the foot as the latter became transformed into a supporting organ." See table III.

TABLE III

ANIMAL	REPORTED BY	OCCURRENCE OF PLANTARIS
Chimpanzee	Beddard	present
Chimpanzee	Hartmann	present
Chimpanzee	Champneys	absent
Chimpanzee	Michaëlis	absent
Chimpanzee	Duckworth	frequently found
Chimpanzee	Author	present
Chimpanzee	Kohlbrugge	absent in 43 per cent
Chimpanzee	Loth	absent in 45.7 per cent
Man	Le Double	frequently absent
Orang	Duvernoy	absent
Orang	Michaëlis	absent
Orang	Hartmann	absent
Orang	Primrose	absent
Baboon	Michaëlis	present
Cercopithecidae	Duckworth	present

The *Soleus* is the largest muscle that enters the Tendo Achillis. It has no tibial head. This is true for the chimpanzee, the baboon, and the macacus. In the chimpanzee, the fibers are inserted all along the inner surface of the Tendo Achillis as far as its insertion. The tibial head was absent in the chimpanzee and in the baboon of Michaëlis. He said that it probably is included in the gastrocnemius lateralis when it is said to be lacking. McMurrich ('04) suggests that the plantaris represents the plantaris profundus III of Amphibian, while Eisler had regarded it as a derivative of the gastrocnemius lateralis.

All the bellies joined to the Tendo Achillis are separate from their origins to their insertions, a condition frequently found in man (Bardeen). Muscle fibers are incorporated with the Tendo Achillis for its whole length. Michaëlis found this to be the case in his chimpanzee. The calf is slim, presenting no more than a slight swelling. This is in sharp contrast to the well developed human calf, which occurs as a result of man's mode of locomotion.

The *Tibialis anterior* has two bellies in both chimpanzee and baboon. In the former, it arises from the anterior external surface of the tibia along its proximal two thirds. One third the distance from its origin, the muscle divides. The internal portion is the larger; its fibers are inserted into the deep aspect of a strong, round tendon, which is inserted into the internal palmar aspect of the distal third of the cuneiform bone. The external portion, whose tendon is half as large as the first one, is inserted into the internal palmar aspect of the base of the first metatarsal bone. This separation of the tendon, inserted partly on the cuneiform, and partly on the metatarsal, has been found as a variation in man (Le Double). In his baboon, Michaëlis, found that the tendon divided just before being inserted upon these bones. In his chimpanzee, it divided at the origin. Beddard and Vrolik described such a separation. Owen describes the tendon to the metatarsal bone as a separate muscle with no homologue in man. Ruge says the division is well known and that Bischoff has found three parts.

The *Peroneus longus* in the chimpanzee arises from the head of the fibula, from the intermuscular septum between it and the

extensor digitorum communis and from the internal border of the fibula down to a point 4 cm. from the distal extremity. It is attached to the first metatarsal bone alone. Primrose describes a double attachment in the orang.

The *Peroneus brevis* arises from the lower half of the fibula between the extensor digitorum communis and the peroneus longus. It is connected with these muscles by septa. Its tendon is inserted into the tip of the tuberosity of the fifth metatarsal bone. From this point, a tendonous slip is continued along the phalanges to be inserted together with the tendon of the extensor digitorum communis into the second phalanx. Owen, in an orang, and Vrolik and Beddard, in the chimpanzee, found this same arrangement. This extended tendon corresponds to the peroneus digiti quinti, a muscle found in the monkeys, which in man is frequently found fused with the peroneus brevis, so that only its tendon of insertion is apparent (Bardeen). Poirier (Morris's "Anatomy" p. 459) considers that all the peroneals are varieties of this muscle (peroneus digiti quinti), which in its most simple form "arises from the distal fourth of the fibula and is inserted by a tendon into the fifth toe."

The *Extensor digitorum longus* in the chimpanzee arises from the tibia and fibula, from the interosseous membrane and the intermuscular septa between it and the tibialis anterior and the peroneals. The tibial belly separates half way down the leg. The muscle fibers are inserted into a tendon, which, after passing through the second tarsal ligament, divides, sending one branch to the second, the other to the third digit. The portions supplying the fourth and the fifth digits do not separate from each other until after passing the tarsal ligament. The tendon for the fifth digit is not free from muscle fibers until it passes the second ligament. This is much like the conditions in man, where there are two main bellies, each of which divides just after passing the ligament. Owen, in his orang, found no tendon to the second digit. Beddard found all four tendons, but the one to the second digit was small.

The *Flexor digitorum longus* (Flexor digitorum tibialis) arises from the whole length of the tibia (fig. 4, *D*); proximally it anas-

tomoses with the popliteus, distally it leaves the bone 4 cm. from its end. The tendon is flat. It does not become entirely free from muscle fibers until after it divides. One tendon is sent to

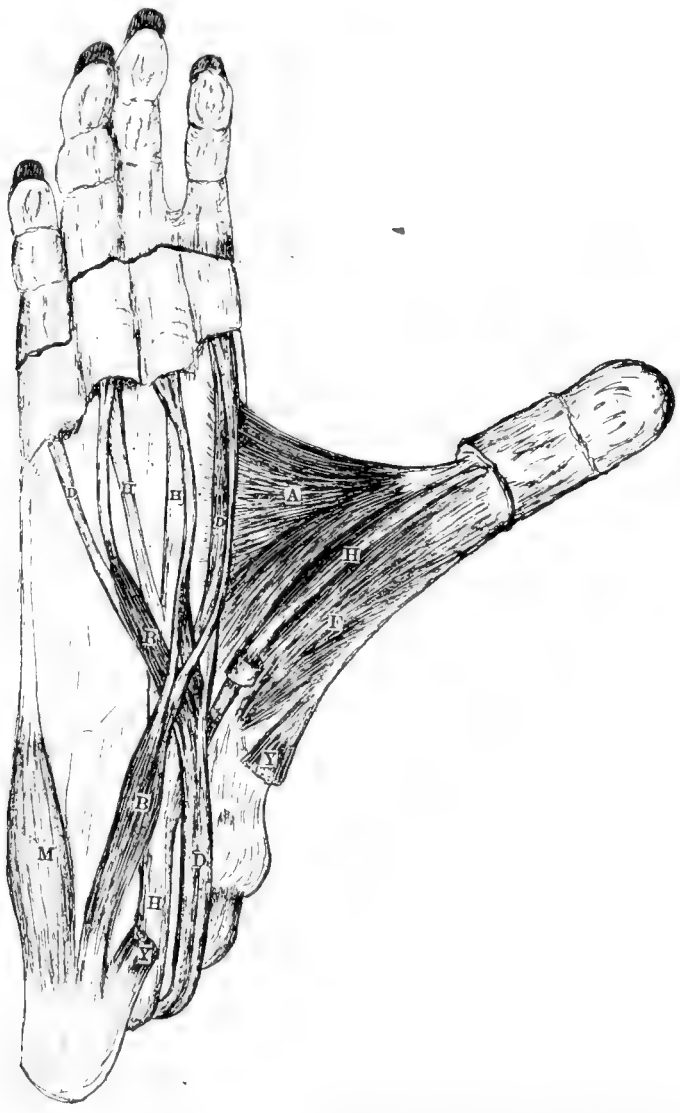

Fig. 4. Dissection of the plantar surface of the right foot of the Chimpanzee. To show the arrangement of the flexor tendons for the digits. *A*, Adductor halluci *B*, Flexor digitorum brevis; *D*, tendons of flexor digitorum longus; *F*, Flexor hallucis brevis; *H*, tendons of Flexor hallucis longus; *M*, Abductor digiti quinti; *Y*, cut ends of Abductor hallucis brevis.

the second digit, the other to the fifth. In the baboon (fig. 5, *D*) the origins and insertions of this muscle are similar.

Champneys found the tendon of this muscle in his chimpanzee fused with the flexor hallucis longus, otherwise it would have

supplied only the second and fifth digits as it does in my animal. In the baboon, he describes the same relations. In Beddard's chimpanzee and orang, this muscle sent perforating tendons to the third, fourth, and fifth digits. In Keith's chimpanzee this muscle supplied the second and fifth digits. Forster said that this muscle supplied the fifth digit in his "Papua neugeborenen" but also united with the flexor hallucis longus to form a tendon, which supplied the other four digits. Owen found three tendons in his orang: first to the second digit (perforans); second to the fourth digit (perforatus); third to the fifth digit (perforans). In my chimpanzee, there arises from this tendon at its division a belly which supplies the perforatus tendon to the fourth digit. However, as no fibers from the common belly are included in this one, I have decided to include it with the flexor digitorum brevis rather than with this muscle. (See table IV.)

TABLE IV

ANIMAL	REPORTED BY	INSERTION OF	
		FLEX. DIG. TIBIALIS	FLEX. DIG. FIBULARIS
Chimpanzee	Champneys	*digits II, V	digits I, III, IV
Chimpanzee	Beddard	digits III, IV, V	digits I, II
Chimpanzee	Keith	digits II, V	digits I, III, IV
Chimpanzee	Michaëlis	digits II, V	digits I, III, IV
Chimpanzee	Duvernoy		no tendon to hallux
Chimpanzee	Author	digits II, V	digits I, III, IV
Baboon	Champneys	digits II, V	digits I, III, IV
Baboon	Michaëlis	digits II, V	digits I, III, IV
Baboon	Author	*digits II, V	digits I, III, IV
Orang	Beddard	digits III, IV, V	digits I, II
Orang	Owen	digits II, IV, V	no tendon to hallux
Orang	Hartmann		no tendon to hallux
Orang	Primrose		no tendon to hallux
Simiadæ	Dobson		digits II, III, IV

The *Flexor hallucis longus* (flexor digitorum fibularis) (fig. 4, *H*) arises from the shaft of the fibula extending from below the insertion of the soleus to a point 6 cm. from its distal extremity,

from the interosseous membrane, and from the intermuscular septum between it and the peroneus longus. The tendon is large and stronger than that of the preceding muscle. It is free from muscle fibers before reaching the plantar surface. Here it divides in half. One branch, passing under a ligamentous band on the inner palmar aspect of the base of the first metatarsal bone, is inserted into the terminal phalanx of the first digit. The other branch divides, sending perforating tendons to the third and fourth digits. The baboon presents the same arrangement (fig. 5, *H*), excepting in the tendon to the first digit, which arises as much from the flexor digitorum longus as from the hallucis. Thus, the two tendons are firmly united at this point, allowing far less independence in the flection of the terminal phalanges of the third and fourth digits. Michaëlis describes a similar arrangement in his chimpanzee and in his baboon. Owen, Hartmann, and Primrose describe no tendons to the hallux in their orangs. Duvernoy found the same lack in a chimpanzee. Dobson says that this muscle supplies the three middle digits in the Simiadæ. The main variations that have been described refer to the relation of the tendon to the first digit, the insertion into third and fourth digits being constant. (See table IV.)

The *Flexor digitorum brevis* will be described as the muscle whose bellies supply the perforated tendons to the digits. In the chimpanzee (fig. 4, *B*), it arises from the calcaneum in close relation to the abductor hallucis brevis and from the tendon of the flexor digitorum longus. The tendon of the second digit arises from the main belly from the calcaneus. The tendon from the third digit arises from a belly that also takes its origin from the "longus" tendon. A few of the fibers from this insertion extend so far back along the tendon that they become continuous with those from the belly of the "longus" that have crept far down the tendon. However, as most of the fibers arise from the tendon just as it divides, it seems fair to have called this belly a separate muscle and not to have described its tendon as one from the flexor digitorum longus. The perforatus tendon for the fifth digit is lacking. Hartmann considers the division arising from the tendon of the flexor digitorum longus as a part of that muscle. He

describes the flexor brevis digitorum in the gorilla as displaying perforated tendons for the second and third digits, while those for the fourth and fifth digits are provided by the flexor digitorum longus.

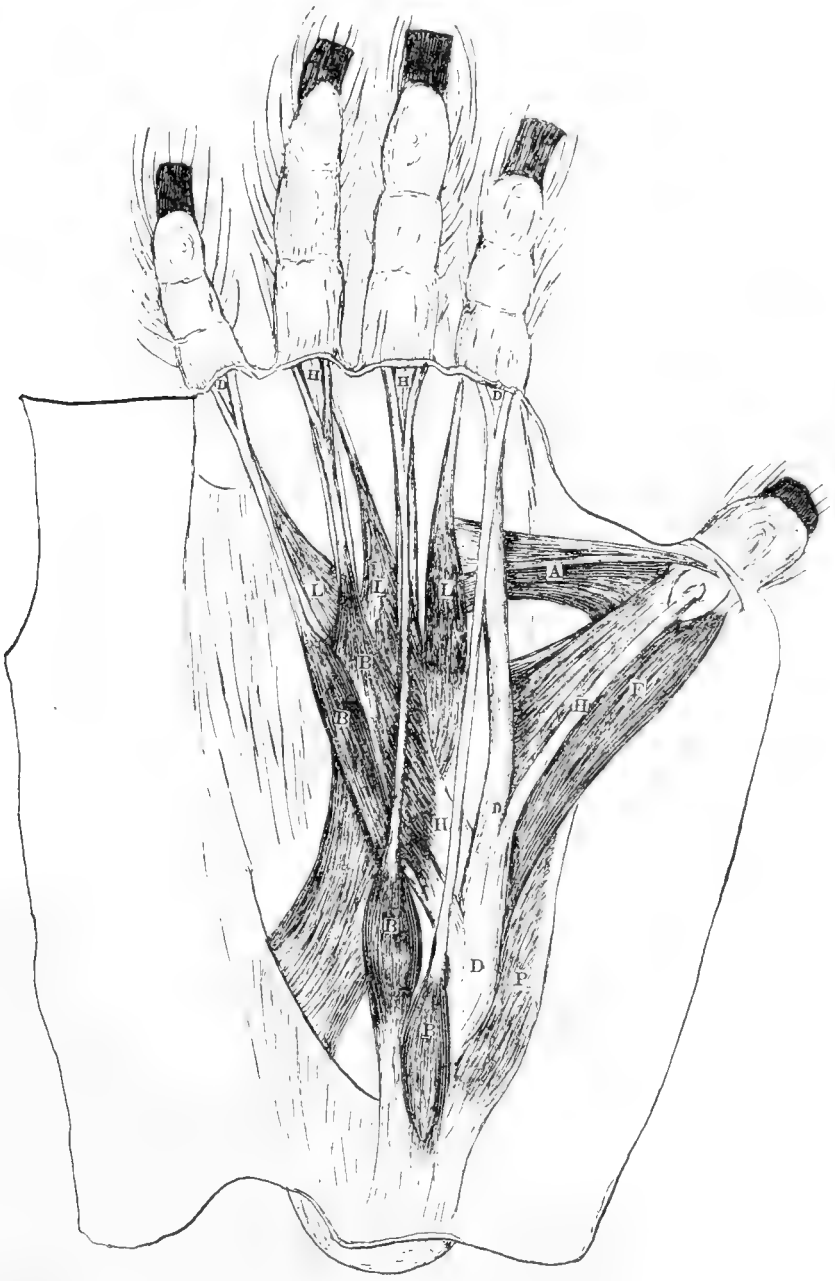

FIG. 5. Dissection of the plantar surface of the right foot of the Baboon, to show the distribution of the bellies of the flexor digitorum brevis. *A*, Adductor hallucis *B*, Flexor digitorum brevis; *D*, tendons of Flexor digitorum longus; *F*, Flexor hallucis brevis; *H*, tendons of Flexor hallucis longus; *L*, Lumbricals; *P*, Abductor hallucis brevis.

The baboon (fig. 5, *B*) shows a greater complexity in the arrangement of the bellies of the flexor digitorum brevis. In general, it arises from the plantar fascia covering the calcaneus, and from the tendons of the flexor digitorum longus and hallucis longus. The first belly arises from the palmar fascia and is situated near the heel. Its tendon is long and slender and is inserted as a perforated tendon into the second digit. The second belly arises from the plantar fascia further from the calcaneus than the first. It swells out as the first one tapers down to receive its tendon. Before its insertion into the third digit the tendon from this second belly is connected by a ladder of muscle fibers to the perforating tendon of the flexor hallucis longus which lies directly below this. The third belly comes partly from the tendon of the flexor digitorum longus for the fifth digit, and partly from the tendon of the hallucis longus for the fourth digit. This belly lies far out from the heel and thus has but a short tendon. The fourth belly has its origin on the tendon of the flexor digitorum longus and in the intermuscular septa between it and the neighboring muscles. This belly is nearer the digits than the third belly.

The flexor digitorum brevis acts more as a set of accelerator for the long flexors than as an independent muscle from the calcaneus. However McMurrich ('07) says that this is an intrinsic muscle, which is homologus with the main mass of the *flexor brevis superficialis str. superficiale.* The greater part of its fibers arise from the tendon of the flexor longus. It may be noticed that this corresponds to the condition found in the chimpanzee, although there was no origin from an hallucis longus tendon.

Champneys describes a muscle in his chimpanzee which is even more complicated than the one I describe. Owen and Beddard found more simple ones in their orangs. The latter found but three tendons. Primrose found four, of which the tendon for the third digit was the strongest; those to the fourth and fifth toes were extremely small. The divisions for the fourth and fifth digits arose as in my chimpanzee, largely from the tendon of the flexor digitorum longus.

All the short muscles of the hallus and pollex are found in both forms with but slight variations from their human homologues.

In the chimpanzee, the flexor pollicis brevis is very closely joined with the oblique head of the adductor pollicis. The opponens is but feebly developed. In both forms the transverse head of the adductor pollicis is well developed and extends well over into the palm.

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STUDIES ON THE CHIEF VEINS IN EARLY PIG EMBRYOS, AND THE ORIGIN OF THE VENA CAVA INFERIOR

DAVID M. DAVIS

From the Anatomical Laboratory, Johns Hopkins University

WITH THREE TEXT FIGURES AND SIX PLATES

INTRODUCTION

The vena cava inferior has always excited a good deal of interest, on account of its unusual history and great importance. When its channel first becomes patent, a great change takes place in the circulatory system of the embryo, and in a very short time—a few hours, in fact—the new vein is carrying practically all the blood from the posterior part of the body. Owing to this circumstance, the vein, by the time the older investigators observed it in stained sections, had already attained considerable size. It was thought, therefore, that an attempt to correlate its earliest origin with the new theory of capillary or capillary plexus anlagen for the vessels, as recently presented by Dr. Evans, would be of interest. The notions formerly held will be understood from the following short historical sketch.

HISTORICAL

In 1888 the result of the work that had been done upon the development of the cava to that time, was summed up by Lockwood and Humphry in these words—"It (the vena cava inf.) commences a little in front of these organs (kidneys) by two blind symmetrical rootlets (the "renal" veins) situated at the base of the mesentery and in front of the aorta. After communicating with both posterior cardinal veins, especially with the right, it runs forward by the side of the aorta, and enters the liver and runs through the substance of that organ to empty into the r. vitelline vein in front of the junction with the ductus venosus arantii. The

vena cava enters the liver through a junction—the caval junction —which that organ previously acquires with the tissues at the base of the mesentery." This is practically the description given by Kölliker, and His seems to confine his attention to the liver veins, commenting only sparingly upon the vena cava. It is noticed that little effort is made to explain the exact means by which the changes take place.

The next important writer on the subject was Hochstetter, whose first work appeared in about 1888, and covered a period of many years. The technique of the time made certain mistakes unavoidable, but his descriptions of the morphological changes occurring in the already formed veins can scarcely be surpassed. Regarding the origin of the cava, he practically reversed the process, speaking apparently, of it as "growing down" from the liver into the Wolffian ridge, and there receiving bilateral veins draining the kidneys and Wolffian bodies.

As is well known, the veins of the very young embryo are quite symmetrical. There are three systems—the vitelline, the umbilical, and the cardinal, each consisting of two bilaterally symmetrical veins, the three of either side uniting at the duct of Cuvier to form the sinus venosus. For reasons as yet unknown, the stomach shifts to the left in development and the liver confines itself mainly to the right side. The stomach, according to F. T. Lewis, presses upon and obliterates the left ala pulmonalis (Ravn) where it extends into the future abdominal cavity, while the liver approaches the corresponding structure upon the right, and appropriates it by sending first capillaries, then hepatic tubules, into it. This bridge between liver and mesenchyme was called by Hochstetter the Hohlvengekröse, and may be designated as "caval mesentery" (Lewis). See fig. 2. Hochstetter's view was that the cava grew down through this caval mesentery, and gave rise to three branches—more properly roots. One of these extended caudally upon the right Wolffian body, the other two caudally and cranially, respectively, upon the left Wolffian body. These two roots upon the left united into a common stalk before crossing, ventral to the aorta, to join their fellow of the right. (These veins were called the vv. revehentes posterior by Hochstetter, and are

identical with the bilateral renal veins of His, and the subcardinals of Lewis.) Communications were set up through the substance of the Wolffian body with the cardinal veins; especially the right; later with the kidneys, and so the ground plan of the adult cava was laid. For more detailed accounts of these conditions as given by Hochstetter, one is referred to his own articles and to various anatomies and embryologies.

In considering these points, we must not forget Zumstein, whose work, criticised adversely by Hochstetter, has enjoyed little recognition. He studied rabbit and mole, as well as human embryos, and while his reconstructions are diagrammatic in the extreme, he observed that the subcardinals exist before the vena cava, and communicate with the liver veins by small vessels. ("Gegen der Kopfende der Urniere treten beiderseits medial und ventral von den Cardinalis kleine Venenlumina aus, welche mit den Kardinal-Venen sich verbinden. Die rechten lassen sich an die Lebergefässe heran verfolgen. Sie sind noch kapillär. . . . In der Leber selbst tritt noch kein deutlicheres Gefässlumen hervor, dass man als cava inferior ansprechen könnte." *Anat. Hefte,* I. 1898, S. 311. The quotation, given also by F. T. Lewis, concerns an embryo mole, 3 mm. in length). As Lewis observes, he did not appreciate the point, and did not push it. It will be seen by this time that the question is almost entirely one of interpretation.

It remained for F. T. Lewis ('01) to describe the subcardinal veins as such. He saw that they antedated the cava, and that they approach very close to the venous spaces of the liver at the time when the cardinal system is "tapped by the hepatic." He did not enter into details as to the exact manner in which the tapping took place, except to remark that "the right subcardinal and hepatic sinusoids approach one another and unite, thus forming a new access to the heart."

Dating from Thoma's paper upon the vascular area of the chick, a new idea of the development of vessels has arisen—namely, that they develop from pre-existing capillaries or capillary plexuses. Thus every advance of the vascular system is preceded by a skirmish line of growing capillary buds. The work of Aeby and of

Baader, as early as 1866, had shown the earliest form of this idea. Although they stated their belief that vessels arose from capillary nets, adverse criticism prevented the adoption of this view for many years. It was advanced by Mall in 1898 and again in 1905, in his work upon the liver. Müller and Rabl have also supported this view, and lately the work of Evans upon this subject has been very convincing. His résumé will give additional information on the subject. (*Anat. Record*, 1908, p. 411).

PRESENT INVESTIGATION

When the time is ripe for the formation of the vena cava, the situation is such that, for its establishment, it is only necessary for a channel to penetrate a very narrow bridge of tissue between two already functioning venous trunks. These two trunks are, first, a branch of the hepatic vein, and second, the right subcardinal vein. To show that this penetration takes place through the medium of capillary outgrowths, is a purpose of this study.

The material used consisted of pig embryos. The following table gives data concerning the specimens reconstructed, and the numbers by which reference is made to them.

Table of embryos used in this work

NO. OF EMBRYO	LENGTH OF EMBRYO IN MILLIMETERS	INJECTED BY	INJECTION MASS	THICKNESS OF SECTIONS IN MICROMILLIMETERS	STAIN
2301.....	7.5	Dr. Evans.	India ink	50	Haematoxylin
2302.....	8.	Dr. Evans.	India ink	100	None
2309.....	9.5	Dr. Evans.	India ink	30	Alum Cochineal
21.....	16.2	writer	India ink	100	None

The reconstructions were made by the profile method, in which tracings upon transparent paper are superposed. This gives a flat reconstruction, from which the drawing is worked up.

A number of other specimens, either in the collection of the Anatomical Laboratory of the Johns Hopkins University, or injected for the purpose, were examined in less detail.

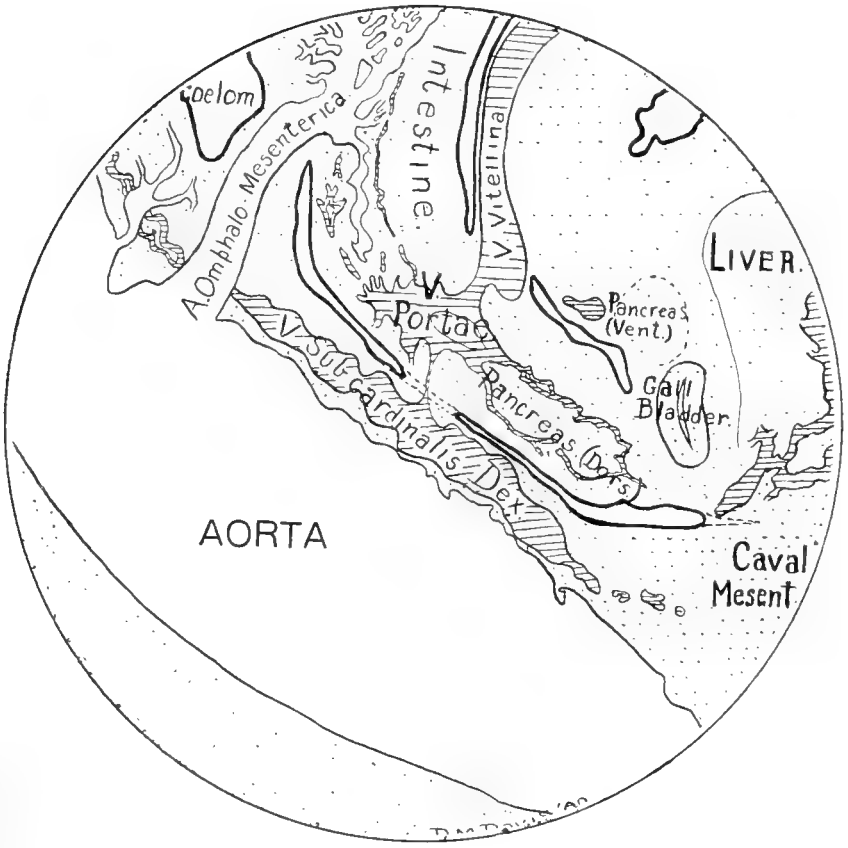

FIG. 1. Embryo 8 m.m. No. 2302. Sagittal section. 100μ. Showing one of the subcardinal-portal anastomoses. The veins are ruled horizontally, the arteries in white, the tissues dotted. At the left is seen one of the small aortic mesonephric branches.

It has been stated above that the vena cava inferior is formed through an inosculation between one of the posterior hepatic veins and the right subcardinal vein. This needs no proof, having been shown clearly by Lewis. An idea of the topography of the viscera at this time will be had by reference to Plates I and II, which represent a 7.5 mm. embryo, and where, it will be seen, the liver is very small, and the subcardinals are fully formed. In spite of the undoubtedly distinct character of these veins, no channels are yet to be made out entering them from the mesentery. This would seem to contradict the theory of Lewis concerning their origin. They have not, however, reached their full extent in an anterior direction, and for that reason approach nowhere close to the liver. Notwithstanding this fact, there is open communication between the subcardinal system and the hepatic system. A channel is seen

extending from the left subcardinal into the omphalo-mesenteric vein (Plate II). This channel lies in the mesentery, and extends in a ventro-mesial direction. It apparently has no relation whatever to the future cava, and seems to be a representative of a more or less numerous system of channels in this region. A later stage may be seen in Plates III and IV, showing an 8 mm. embryo. Here the channels are multiple, and pass from both subcardinals into the omphalo-mesenteric vein. One of them is seen in section in fig. 1. The one figured here is of almost capillary dimensions. In this specimen, similar vessels are seen in the mesentery caudal to the inferior mesenteric artery, and joining the subcardinal system to the inferior mesenteric vein. All these channels appear later to be completely obliterated, and take no part in the formation either of the vena cava, in the upper part, or of the veins of Retzius, in the lower part. Their significance is conjectural, although they seem to convey a considerable amount of blood to the liver. I have been able to find no previous reference to them. It is interesting to note that, in the adult human, veins may pass from the duodenum directly to the vena cava, as a rare anomaly.

The venous changes in the liver during its growth are well described by Hochstetter, whose work has been amplified by recent writers. The growth of the liver itself, in the region with which we are concerned, is well discussed by Lewis, whose conclusions have been referred to. The area designated in Plate III, as "Site of Vena Cava Inferior," is shown as it appeared in section in fig. 2. One sees the liver just after it has touched and fused with the "caval mesentery." At the top of the drawing are the liver cells and liver capillaries, growing downward into the caval mesentery. In advance are capillary sprouts—extending out toward other capillary sprouts from the somatic area. To understand the origin of these second sprouts, another system must be studied.

In a 7.5 mm. embryo, Plates I and II, the subcardinals, at their anterior ends, are still in the growing state. Numerous fine, irregular capillaries extend forward, both in and beyond the Wolffian body. As they pass beyond the confines of the gland, they grow into the adjacent tissue, which is, on either side, the abdominal extension of Ravn's ala pulmonalis—on the right side we may

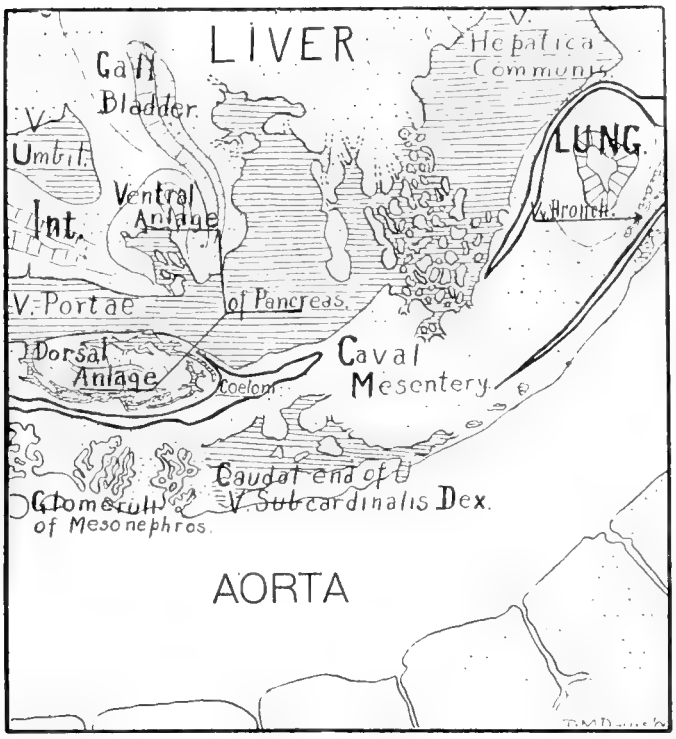

FIG. 2. Embryo 8 mm. No. 2302. Sagittal section. 100μ. Showing the capillary sprouts in the caval mesentery. The veins are ruled horizontally, the arteries in white, the tissues dotted. Within the liver the capillaries are sinusoidal in character. The capillaries of the more ventral parts of the liver are not drawn in.

speak of it as the "caval mesentery." Other capillaries also grow into these ridges—across the front of the aortic roots from the two posterior cardinal veins, from the sinuses of Cuvier, and from the bronchial veins in the developing lungs. At this point the symmetrical development ceases, and the two ridges have very different histories. On the left, the stomach lies upon the ala of that side, but no fusion occurs, and the ridge later disappears. The vessels of the stomach wall and oesophagus anastomose through the dorsal mesentery of the oesophagus with the anterior branches of the subcardinals. This suggests the oesophageal collateral path in portal obstruction. On the right, however, the ridge remains, as the "caval mesentery"—consequently the branches of the r. subcardinal draining it are somewhat larger than those on the left. This is the condition seen in Plates III and IV. Depicted in Plate IV are the anastomoses between this cephalad extension of the subcardinal and the stomach veins; and in both

plates vessels extending still farther and draining the oesophagus and other tissues between the two aortic roots. This area about corresponds with the posterior mediastinum in the adult. In Plate III, the slightly greater size of that portion of the right subcardinal draining the caval mesentery is apparent. Now, returning to fig. 2, we can understand the situation. The factors are, first, the hepatic capillaries reaching down into the caval mesentery, draining their blood into the hepatic veins, vena hepatica communis, and sinus venosus; and, second, the subcardinal capillaries, pushing out through the caval mesentery toward the liver. The consequence is obvious; growing toward each other, they come into contact, and inosculate. It was at this period of development that Zumstein made his observation—and Plate V shows the condition when the new channel has become somewhat enlarged. The sectional appearance is shown in fig. 3, where it is seen that hepatic tubules still obstruct the caval lumen to some extent. The changes at this period, both progressive and regressive, are very rapid. The division of the posterior cardinals into anterior and posterior portions has already commenced. It soon becomes complete (Plate VI), all the blood from the posterior portion of the body pouring through the new channel. The enlargement is rapid, the condition shown in Plate VI (16.2 mm.) being fully reached in a remarkably short time—about two days; a closer estimate of the period of change is difficult to arrive at in the case of pigs. In Plate V (9.5 mm.) the ultimate course of the complete cava is well seen—it is formed by the subcardinal for about half the length of the mesonephros; from there caudad it is a part of the right posterior cardinal. The designations of pars hepatica, pars subcardinalis, and pars cardinalis, are self-explanatory. Of course, the relative length of the pars subcardinalis becomes greatly reduced as the liver grows downward and the thorax expands.

The connections of the veins of the caval mesentery, which aided in the formation of the cava, remain as they were originally—as a result we have a good sized vein flowing from the oesophageal (posterior mediastinal) region into the cava just where it bends ventrally to enter the liver (Plates V and VI). The network of

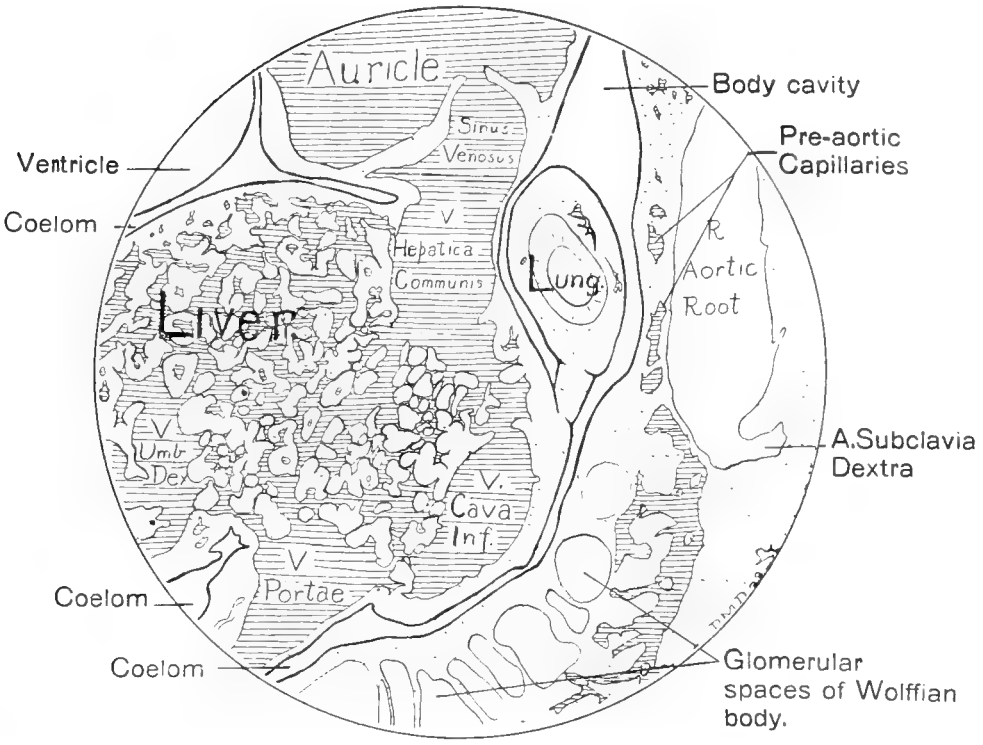

FIG. 3. Embryo 9.5 mm. No. 2309. Sagittal section. 30μ. Showing the lumen of the newly-formed vena cava inferior still partly obstructed by liver cylinders. Just ventral to the aortic root are to be seen the forward prolongations of the subcardinal communicating with the peri-oesophageal plexus.

veins surrounding the oesophagus has been called in this paper the peri-oesophageal plexus. In tracing the history of this peri-oesophageal plexus, it will be noted, first, that, while the subcardinal-portal anastomoses mentioned above, which lie caudal to the circum-intestinal rings of the portal system, become completely obliterated before the vena cava appears; second, these other anastomoses, by way of the peri-oesophageal plexus and gastric veins, remain patent during the entire period, and do not show any special enlargement. This would indicate some inherent advantage in the route through the caval mesentery. These connections through the gastric veins are also noteworthy as the forerunners of the vessels making up the gastro-oesophageal collateral channel in portal obstruction.

It would, then, appear that the vena cava is no exception to the rules governing the development of other vessels—having its anlage in capillary sprouts at the critical point in the caval

mesentery. Since the subcardinal takes so prominent a part in the synthesis of the cava, its own origin must be of interest. Lewis spoke of it in rabbits as the result of longitudinal anastomoses between small veins entering the cardinal from the mesentery and mesonephric tributaries. This must be disputed, at least in the pig, since in a number of early injected specimens, the writer has seen distinct subcardinals, having no tributaries whatsoever from the mesentery. These tributaries appear later. At the earliest stage, the mesonephros is seen filled with a network of capillaries springing from the cardinal vein; soon some of them ventral to the mesonephric arteries dilate and form the longitudinal channel of the subcardinal. The paper of Grafe, written of the chick's mesonephros, bears eloquent witness on this point, even though he supposed some of the vessels to arise in situ. Thus we have the history of the caval channel arising everywhere from capillary anlagen. At later stages, numbers of channels are formed in the same way in the mesonephros. An example of them is a great ventro-lateral vein, appearing in the different drawings. It is seen in pigs as small as 7 mm., and draws blood from the periphery of the gland at its ventro-lateral angle. It retains connections with the anterior portion of the posterior cardinal vein until a very late stage, when, with the regression of the Wolffian body it disappears, taking no part in the formation of any important permanent vein.

The manner of origin of the various trunks of the vascular systems has long interested embryologists, and two sorts of explanation have been advanced: according to one idea, arteries and veins grow out as such to their end territories, and it will be recalled in this connection that Hochstetter spoke of the vena cava as "growing down" into the region of the Wolffian body. According to the other idea of the development of the vascular system, arteries and veins exist originally in the form of simple capillaries, usually in the form of a typical plexus. From this netlike or plexiform condition the trunks of the adult are differentiated. The latter idea, suggested by Aeby and Baader, has recently been proven to be the correct one in the history of various vessels, Evans having been able to show that the very largest

trunks, such as the pulmonary, the carotid, and the limb arteries can be traced to their origin from a capillary plexus. From the same injections Miss Smith has shown clearly that the thoraco-epigastric vein is formed from certain early body wall capillaries.

It is evident that the final act in the formation of the vena cava inferior furnishes another clear instance of a capillary plexus ancestry, for this portion of this great vessel. It has already been pointed out that Zumstein deserves the credit of first having seen these capillary connections, which are the precursors of the cava here, and more recently F. T. Lewis has described and figured the invasion of the caval mesentery by hepatic sinusoids. No one, however, has interpreted these phenomena in the cava's development in accordance with the theory of the development of the vascular system from capillary plexuses. These injections show conclusively that here we are dealing with the fusion of two capillary plexuses, one, as already mentioned, from the hepatic vessels, the other from the cephalic portion of the right subcardinal vein; and the writer has chanced upon the stage in which the fusion has just taken place, and in which, consequently, for the first time a complete vascular path is afforded from the subcardinal to the hepatic channels.

CONCLUSIONS

The conclusions drawn from this study are as follows:

1. Open connections exist between the subcardinal veins and portal system at an early stage, but they reach their maximum and are obliterated before the vena cava is formed.

2. At the time that subcardinal capillaries form the anlage of the cava in the region of the caval mesentery they have previously proliferated in a cephalic direction surrounding the œsophagus; consequently before the cava is formed this peri-œsphageal plexus drains into the cephalic tip of the v. subcardinalis, and with the formation and rapid enlargement of the cava it naturally comes about that this plexus is now drained into the latter vessel.

3. At the critical period in the history of the vena cava, its channel is definitely formed by the fusion in the caval mesentery of capillary sprouts from the hepatic and subcardinal vessels, respec-

tively. Circulatory conditions, as yet not clearly understood, almost immediately force upon these minute capillaries a speedy and tremendous enlargement, so that they quickly constitute the vena cava inferior in this region.

My thanks are due Professor Mall, for the suggestion of this research as well as for his generous supervision. I also wish to express my appreciation of the constant interest and invaluable suggestions and criticisms of Dr. Evans.

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PLATES

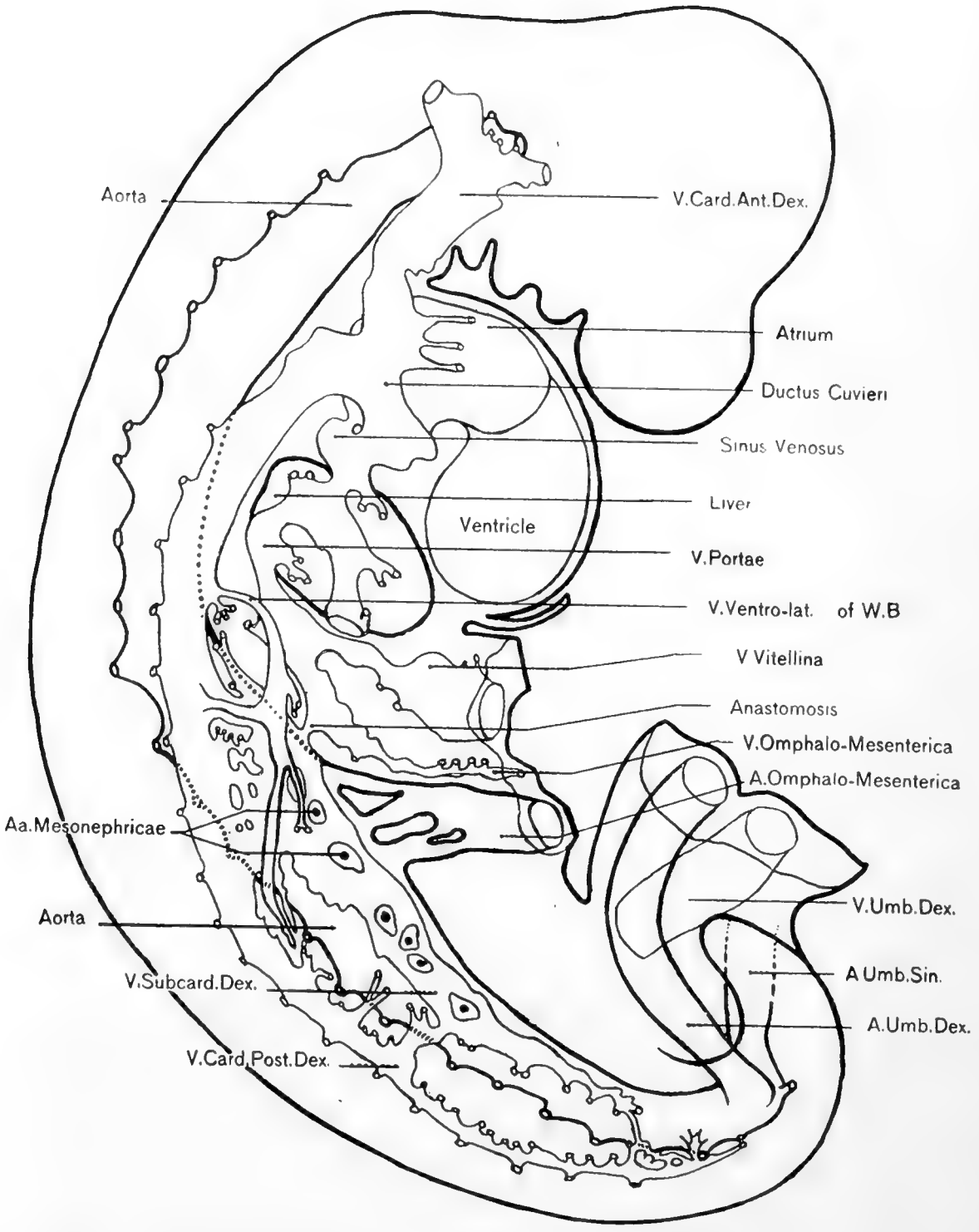
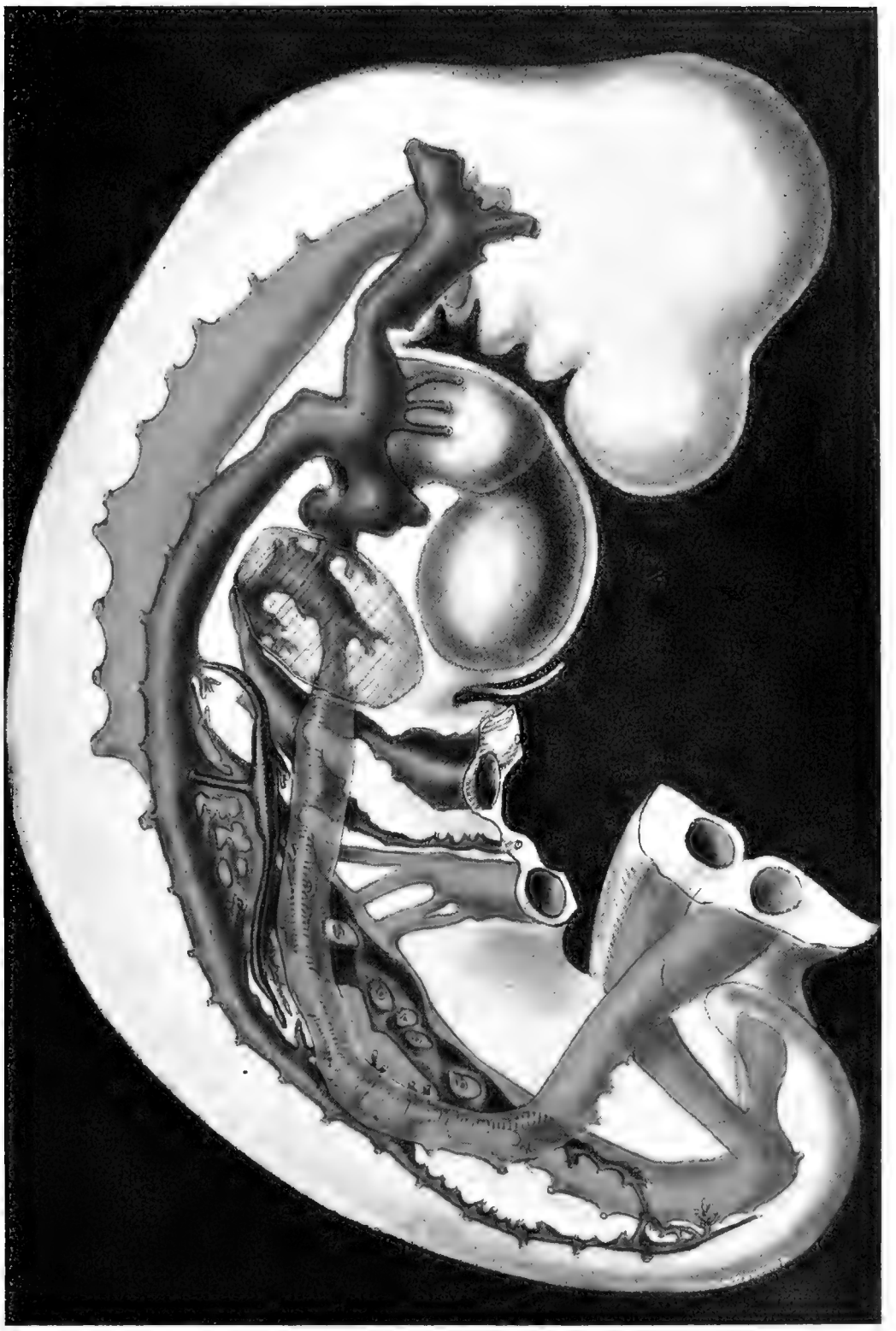


PLATE 1. Pig embryo, length 7.5-8 mm. No. 2301. Reconstruction of venous system of right side, viewed from the right. The right umbilical vein is represented as transparent, with the subcardinal, cardinal, etc., shining through. Note the large size of the anastomosis between the subcardinal and portal veins; fenestrated character and large size of the right subcardinal; and the ventro-lateral vein of the mesonephros. × 19.0.



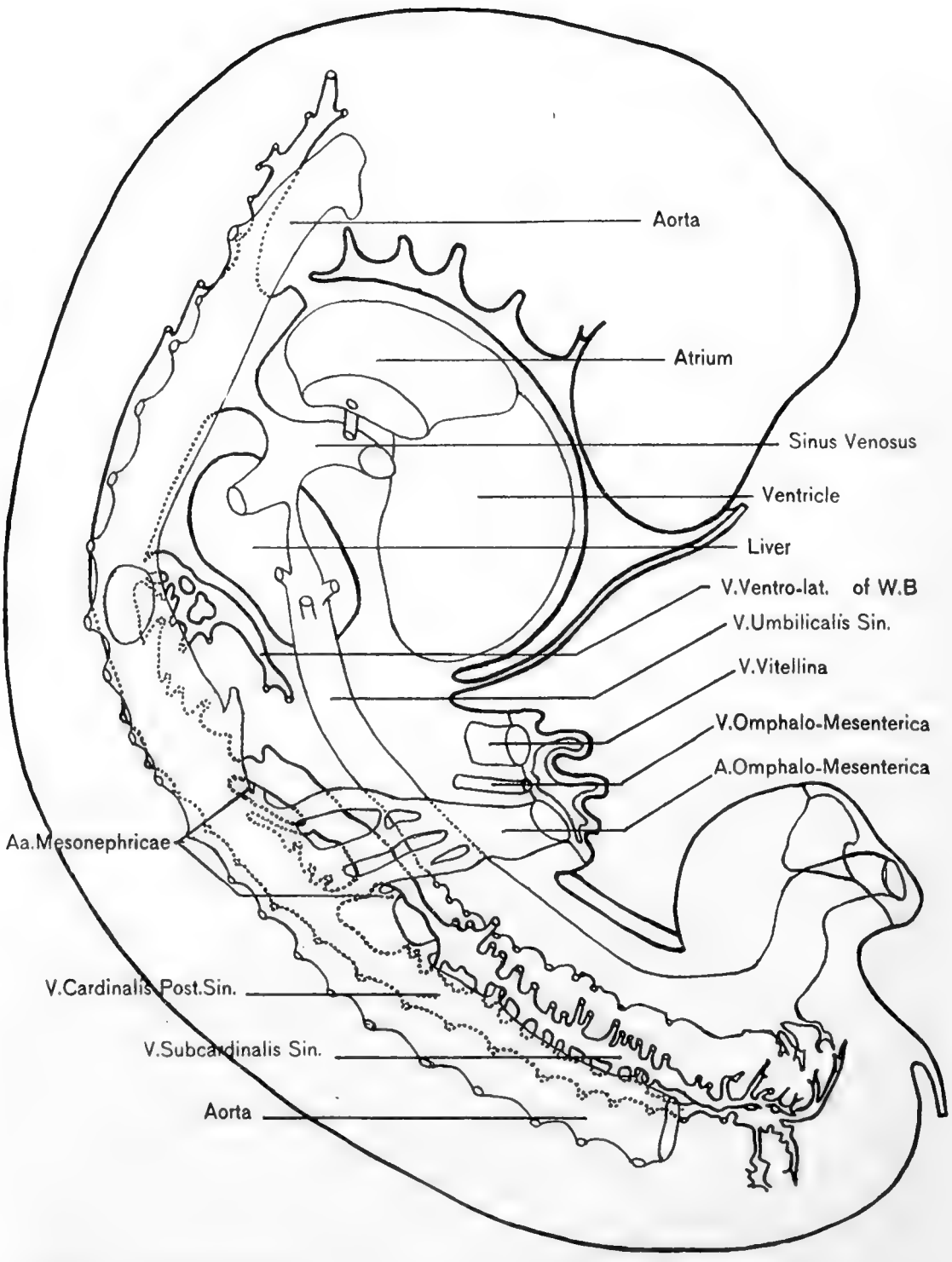
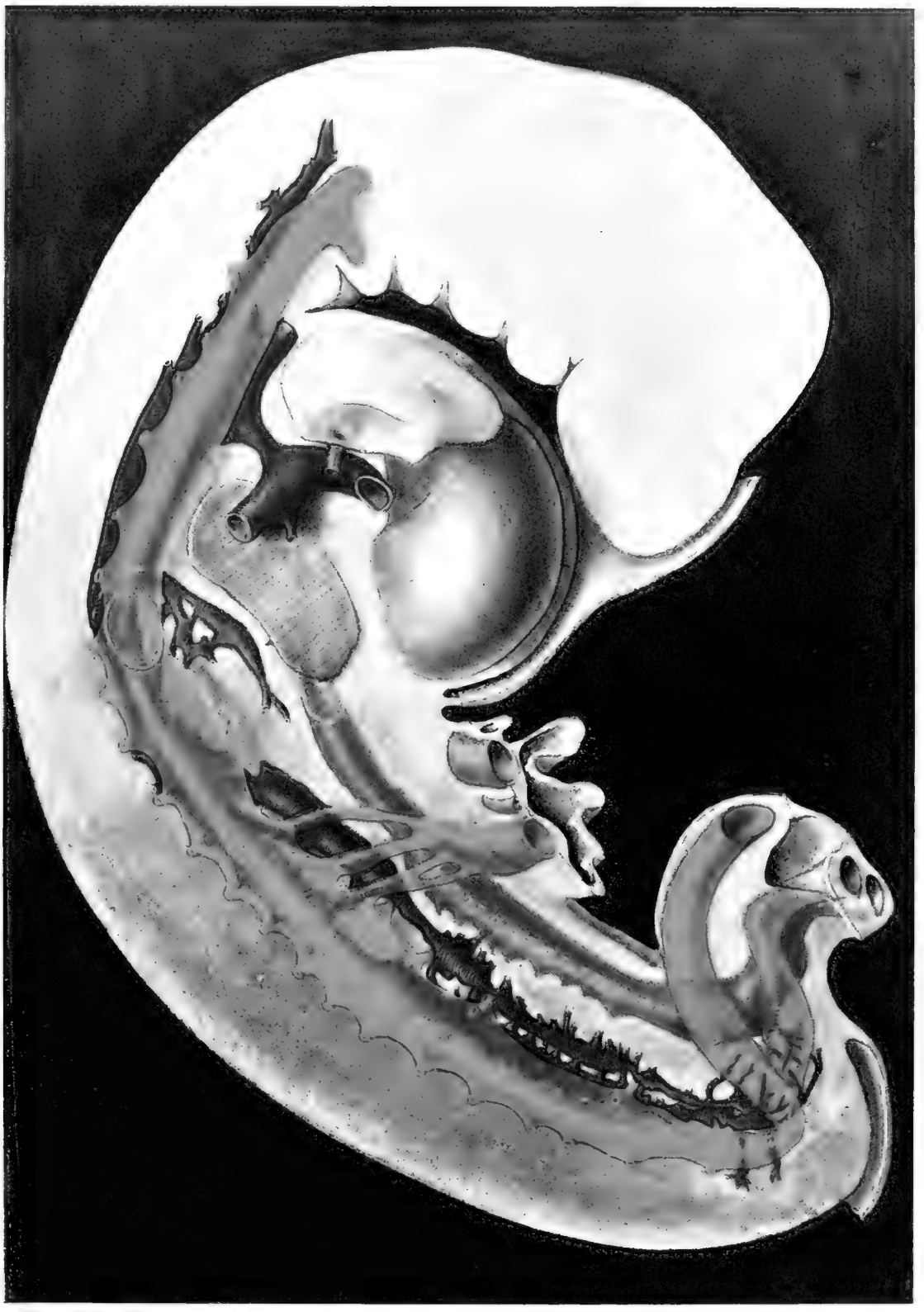


PLATE 2. Same Embryo as Plate 1. Reconstruction of venous system of left side, mesial view from the right. The aorta is represented as transparent, with the cardinal shining through. The white interrupted line gives the outline of the left Wolffian body. The caudal part of the embryo is somewhat twisted, so the small vessels entering the subcardinal caudal to the mesenteric artery are lateral (mesonephric) not ventral tributaries. × 19.0.



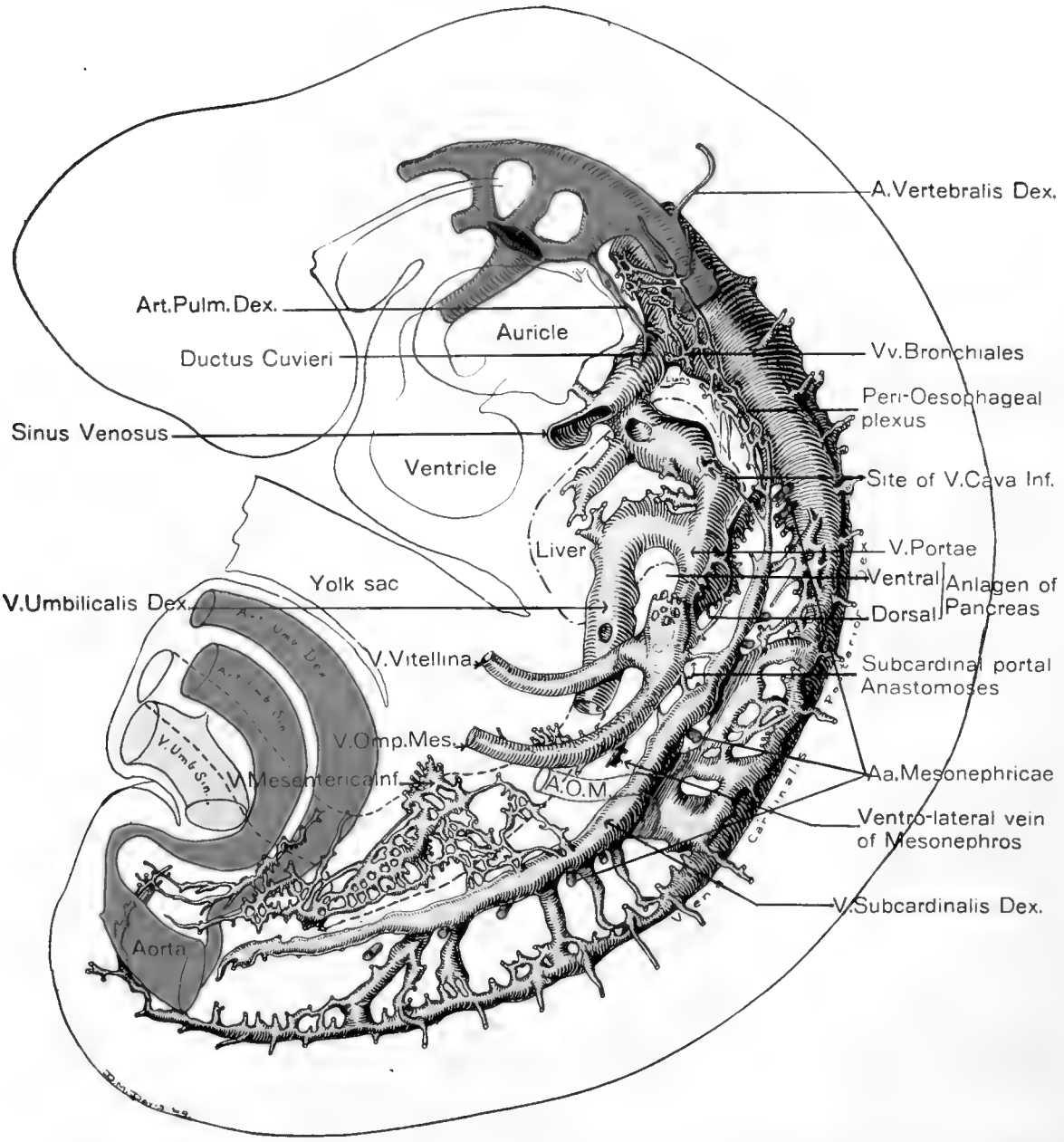


PLATE 3. Pig Embryo, length 8-8.5 mm. No. 2302. Reconstruction of venous system of right side, mesial view from the left. The aorta, excepting its two extremities has been omitted. Text fig. 3 represents a section at the point "Site of V. Cava Inf." The position of the omphalo-mesenteric artery is shown by dotted lines. × 15.5.

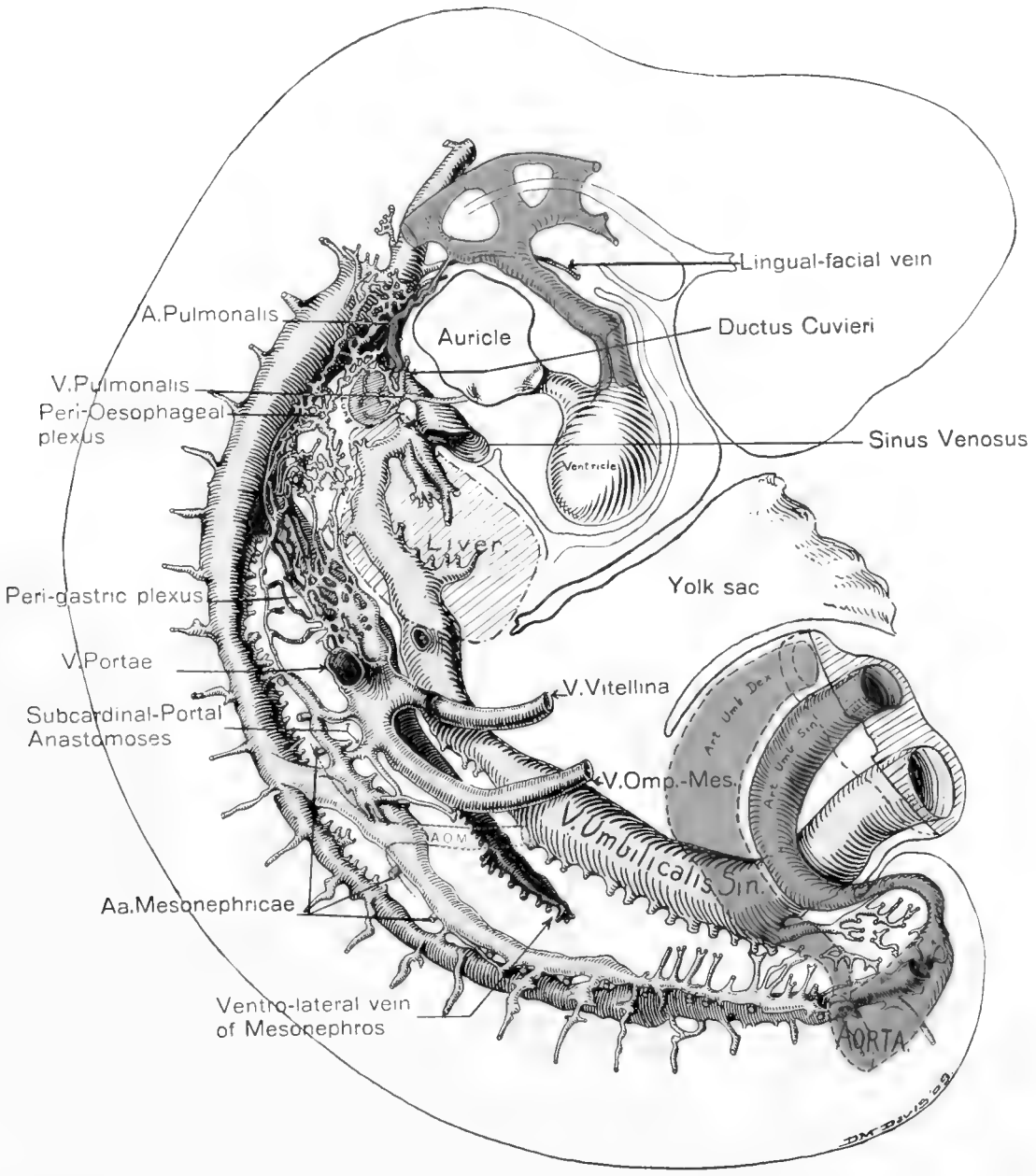
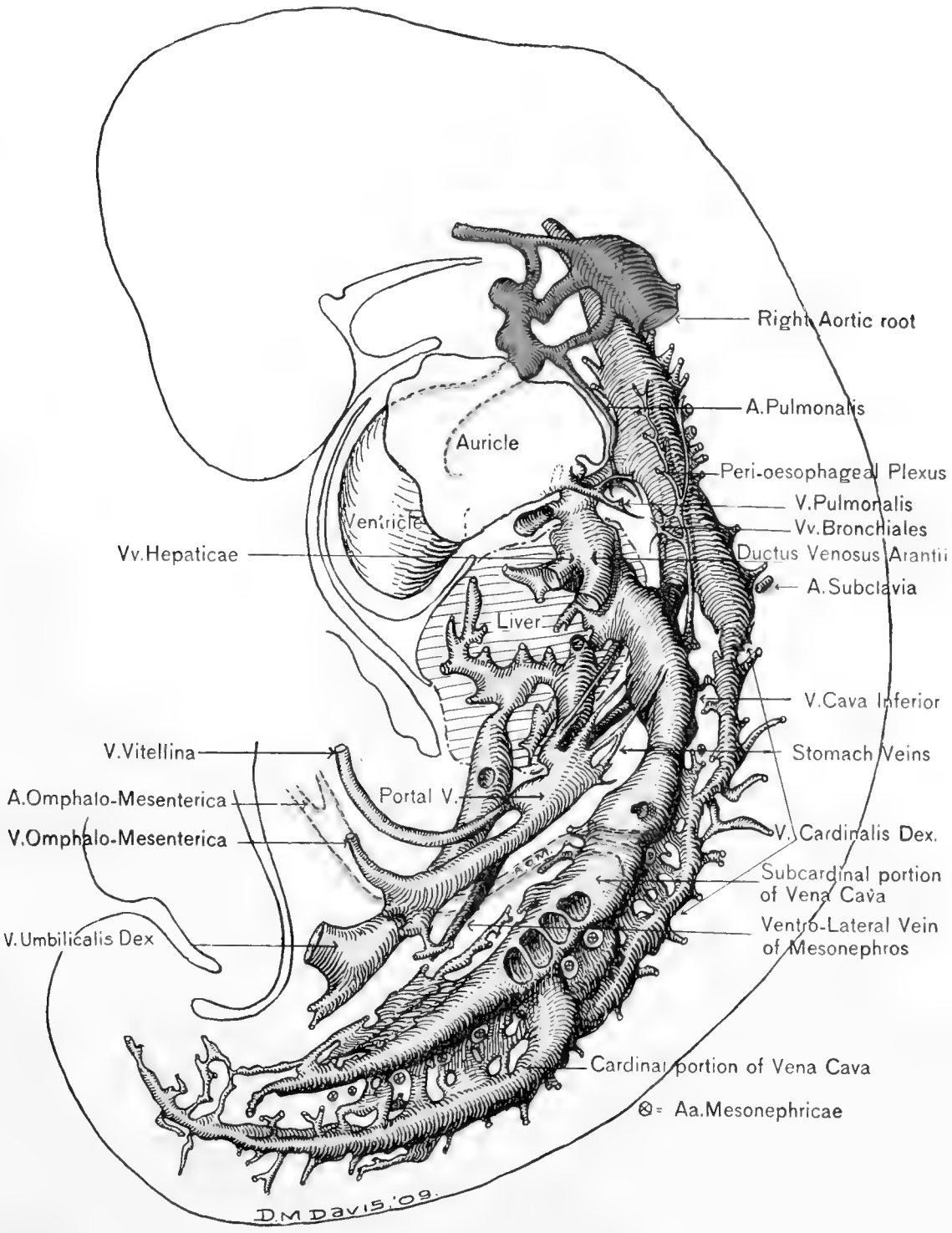


PLATE 4. Same embryo as Plate III. Reconstruction of venous system of left side, mesial view from the right. The aorta, excepting its two extremities, has been omitted. The position of the omphalo-mesenteric artery is shown by dotted lines. The portal vein has been cut off where it passes to the right dorsal to the intestine. × 15.5.

PLATE 5. Pig Embryo, length 9.5 mm. No. 2309. Reconstruction of venous system of right side, mesial view from the left. Only the upper end of the right aortic root is shown. The ductus venosus has been cut off shortly before entering the common hepatic vein. The position of the omphalo-mesenteric artery is shown by dotted lines. The head of the Wolffian body would extend as far up as the level of the subclavian artery. The connections between stomach veins and peri-oesophageal plexus are not shown. × 15.2.


Right Aortic root
A.Pulmonalis
Auricle
Peri-oesophageal Plexus
V.Pulmonalis
Ventricle
Vv.Bronchiales
Vv.Hepaticae
Ductus Venosus Arantii
A.Subclavia
Liver
V.Cava Inferior
V.Vitellina
Stomach Veins
A.Omphalo-Mesenterica
Portal V.
V.Omphalo-Mesenterica
V. Cardinalis Dex.
Subcardinal portion of Vena Cava
V.Umbilicalis Dex
Ventro-Lateral Vein of Mesonephros
Cardinal portion of Vena Cava
⊗= Aa.Mesonephricae
D.M Davis '09.

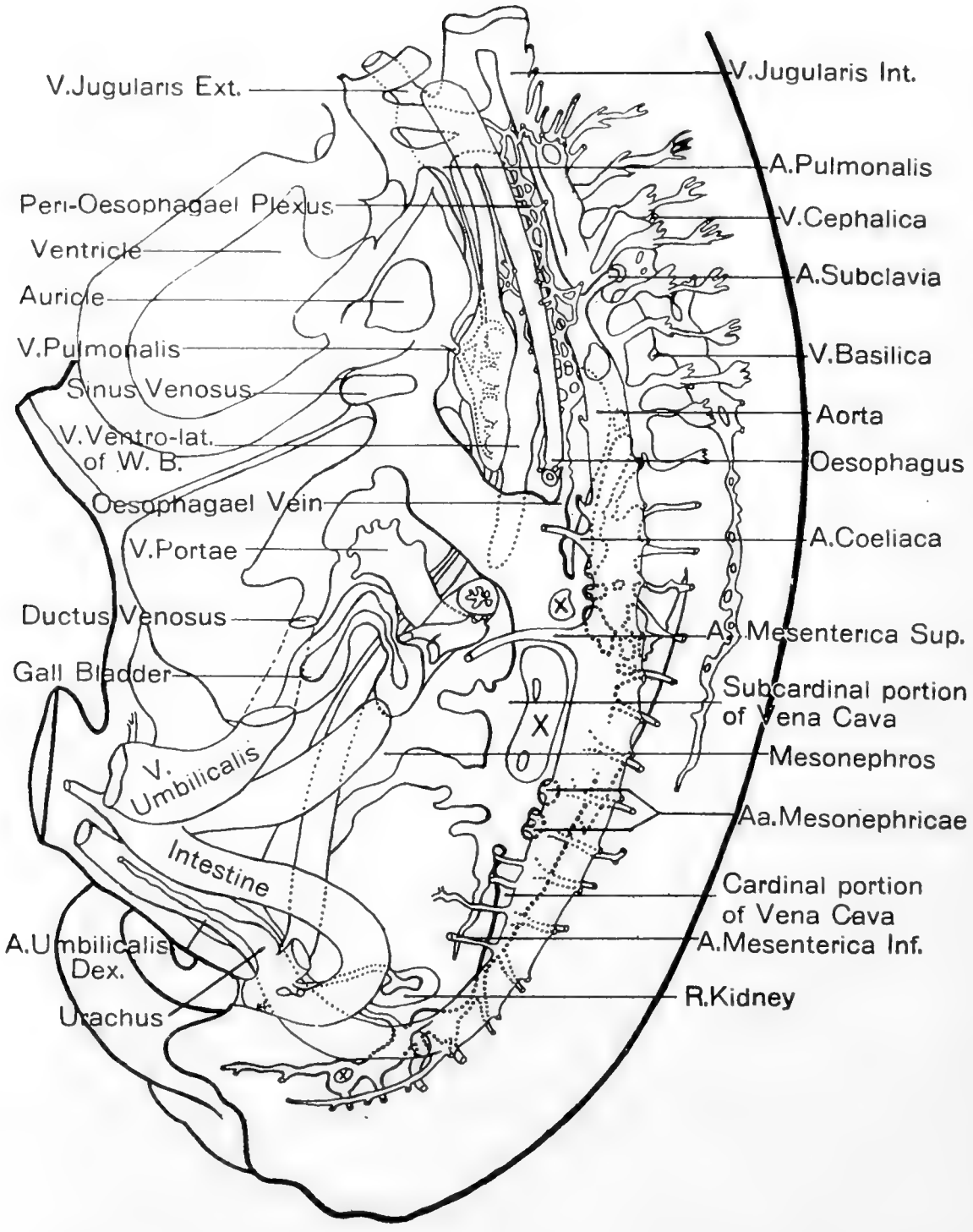
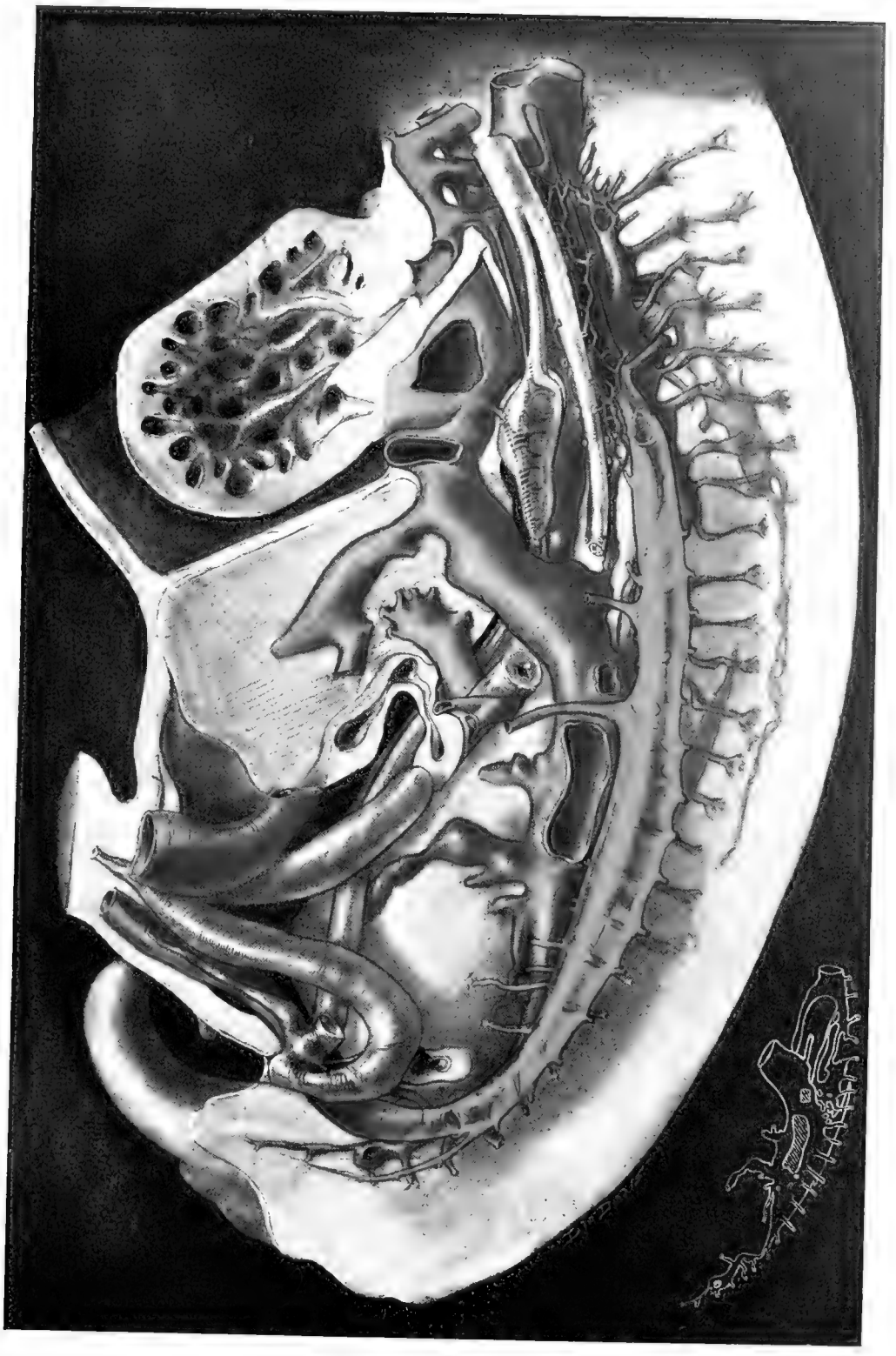


PLATE 6. Pig Embryo, length 16.2 mm. No. 21. Reconstruction of venous system of right side, mesial view from left. The omphalo-mesenteric inf. mesenteric and coeliac arteries have been cut off not far from their origins. A portion of the umbilical vein has been removed. The stomach has been removed from its position, over the vena cava. The right kidney is shown in section. The stumps of the left Wolffian duct and of the left ureter are shown. The three cross anastomoses of the venous system are shown at *x*, *x*, *x*. × 11.1.



ON THE HISTOGENESIS OF GASTRIC GLANDS

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WITH TWENTY-SIX FIGURES

CONTENTS

I. INTRODUCTION

This paper represents an attempt to throw some light on the fundamental processes in the cytomorphosis of mammalian gastric glands. A study of the literature convinces one that, in spite of numerous researches, there still persists a marked lack of agreement even as to the broader outlines, which becomes, with regard to more minute details, a chaotic mass of contradiction, partially referable to the normal generic variations in the forms used, and partially, perhaps, to misinterpretation.

It was decided, on the following grounds, to limit this investigation to the study of a single species, the pig:—First, theoretical considerations of a general, embryologic nature, lead one to infer that, aside from slight generic differences, the same essential processes are probably concerned in the gastric adenogenesis

of the whole mammalian series. Second, one may disentangle from the generally confused data of the literature, certain concordant results, which confirm the validity of this assumption as to the essential unity of the process throughout mammals. Third, and very obviously, a limitation to one form means the possibility of more detailed work than is practicable in a comparative study. The present need seems to be for minute work, like that of Toldt on the cat, but utilising valuable, recently developed technique, and also one old technique, evidently very often neglected nowadays, namely the patient use of serial reconstructions in place of the easier reliance on occasional isolated sections. Finally, and herein lies the proximal motive in selecting this particular species—an abundance of fresh material of every stage has been readily procurable.

I wish to express my sincere gratitude to Dr. R. R. Bensley, at whose suggestion this work was undertaken, and under whose guidance it has been carried on. Without the aid of certain methods devised by him, much of the work on cyto-differentiation would have been technically impossible. I am indebted to Miss Katharine Hill for the accurate drawings.

II. HISTORICAL

Essentially all authors agree that the gastric epithelium is derived from the endoderm. Brand '77, Sewall, '78 and Kölliker, '84 describe a transformation of the original single endodermic layer into a stratified epithelium, which later, by some unexplained mechanism, again becomes simple. All recent writers who mention the subject at all (Toldt '81, Salivoli '90, Ross '03) agree that the epithelium remains simple from the first, although the disposition of nuclei in several planes simulates stratification; of course this does not apply to areas which become stratified and remain so, *e. g.*, the left compartment of the field mouse stomach (Töpfer '91), or the pars oesophagea.

Concerning the origin of the rudimentary gland tubules, there is great diversity of opinion. The various views may be classified under three general groups, depending on whether the tubules

are believed to develop (1) from the surface epithelium, (2) from special embryonic cells interpolated between the basal parts of the ordinary surface epithelium, or (3) from mesodermic cells, such as leucocytes.

Group 1. Of those who describe the glands as originating as downgrowths of the surface epithelium, several have considered the all-important factor in early adeno-genesis to be irregular growth of the mesoderm, first manifesting itself as upgrowths, in the form of either villi or ridges. These coalesce or intersect, thus giving rise to intervening cul-de-sacs, the rudimentary glands. Of course the surface epithelium multiples to keep pace with the increasing area of mesoderm it must cover, but, by convention, this activity is considered secondary to that of the mesoblast. (For details *vide* Laskowsky '68, Schenk '74, Brand '77, Sewall '78, Baginsky '82, Kölliker '84, p. 360, Negrini '86.) Observations recorded in part 2 of this paper explain, we believe, the discrepancies of opinion regarding the exact nature of these mesodermic irregularities, *i. e.*, whether villi, papillæ or ridges. Kölliker ('52–'61) Toldt '81 (pylorus of cat) Patzelt '84, Griffini and Vassale '88, and Salivoli '90 believe that the epithelium displays the initial activity, and that the glands may be said to have an intra-epithelial origin, irregular growth of mesoderm later becoming a factor in bringing the glands to their definitive form. Thus, Kölliker, in '52–'61, described the downgrowth of solid plugs of epithelium, which later acquire a lumen; he discarded this view ('84), nor has it since been advocated. Griffini and Vassale describe the gland as originating from the surface epithelium, as a funnel-shaped evagination, sinking down into the mesoderm. The deepest cells, being in continual mitosis, constitute an apical growth point. Only at a comparatively late stage does unequal mesodermic growth participate. According to Salvioli ('90, rabbit), at an early stage (4 cm.), while the basement membrane is yet perfectly level, intersecting ridges of epithelial cells rise above the general level of the epithelium. The intervening depressions are the rudimentary tubules. Later (6 cm.), these epithelial ridges are reinforced from below by cores of connec-

tive tissue. Toldt ('81, cat) describes a similar process in the pyloric region. "The process is wholly confined to the epithelial layer." Patzelt ('84) describes the same process in the formation of the glands of the large intestine.

Group 2. Toldt (81 cat) working on the fundic region, and Ross (1903, pig), describes the rudiments of the glands as large, coarsely granular, eosinophile cells interpolated between the basal parts of the surface epithelium. Each divides into a cell group, which, by central liquefaction acquires a lumen, the latter secondarily coming into communication with the surface. Toldt is sure these cells are of epithelial origin, but believes they at no time reach the surface, being always shut off from the latter by the overhanging distal ends of the tall pyramidal surface epithelium; he suspects that they arise from young Ersatzzellen His Ersatzzellen have almost certainly been shown by the work of Stöhr (1882) and Bizzozero (1888) to be "Wanderzellen." Griffini and Vassale maintain that Toldt's figures and text harmonize remarkably with their own findings (V. supra), except that Toldt, through use of oblique sections, erroneously concluded that these primary gland cells do not reach the surface, and that their lumen is thus not at first continuous with the stomach lumen. Griffini and Vassale found many such groups with lumina apparently shut in on all sides, but reconstruction always demonstrated continuity with the stomach lumen from the first.

Group 3. Sewall ('78) believes that, once the original hypoblast has differentiated into ovoids (parietals) and central (chief) cells, new ovoids "*originate*" by differentiation of mesodermic corpuscles. In embryo cats (13 cm) mesoblast cells are found, presenting all transitions from connective tissue corpuscles to parietal cells (Toldt says that Salvioli simply cut the eccentric parietals tangentially from adjacent tubules, and hence misinterpreted them to be cells lying free in the lamina propria). The reader must be referred to the recent work of Strecker ('08), which is too elaborate to be reviewed here. His conclusions are diametrically opposed to mine. The diversity of these views as to the early formation of gland tubules make evident the need of further

investigation of the two separate problems involved: (1), from exactly what cells do the glands arise (2), to what extent are irregular growth of mesoderm and epithelium, respectively, factors.

Compounding of the tubules is unanimously described as due to the upgrowth, at the bottom of the simple tubules, of partitions which never reach the surface. Toldt, '81, believes that these are, at first, proliferating solid ridges of epithelium, later reinforced from below by a core of mesoderm. Sewall, '78, Negrini, '86, and Ross, '05, hold that the upgrowths contain mesodermic cores from the first. According to Salvioli, '90, the process described in the original formation of the foveola is repeated at the bottom of the foveola. By mitosis, intersecting epithelial ridges arise; later these are reinforced from below by mesodermic cores.

Toldt, Salvioli, Griffini and Vassale (*opera cit.*) also describe a second method of compounding by "side-buds." Lateral diverticula appear, originating through local proliferation of a cell-group, which starts from a single parietal cell. I have confirmed this for the pig (*vide* part IV).

As to cytologic differentiation, Laskowsky, Sewall, Toldt, *et al.*, describe an early departure of the tubule cells from the surface epithelial type, the former becoming polygonal or ovoid, their cytoplasm granular, and their nuclei large and vesicular. Toldt, '81, Negrini, '86, and others demonstrate that parietals appear in the depths of the tubules by differentiation of the embryonic tubule cells. Bensley, '03, finds that mucus cells appear in the pylorus and along the lesser curve in the 6 cm. pig. Parietals appear in cardia and fundus at 7.5 cm., inter-cellular ductules being present from the first. Zymogenic (serous chief) cells appear, at about 21 cm., at the bottom of the fundic glands. A few, as Sewall, '78, and Strecker, '08, believe in a possible mesodermic origin of parietals, or of the whole gland anlagen, either from leucocytes or from mesodermic corpuscles, fibroblasts etc.

III. MATERIAL AND TECHNIQUE

The embryos were obtained in an absolutely fresh condition through the kindness of Swift & Company, of the Chicago Stock

Yards. Half a minute after the instantaneous death of the mother, by cerebral concussion, the uterus was cut out, and immediately opened. The embryos were removed, and their greatest length in the natural attitude along a straight line (Minot's System. *Vide* Minot, '03, p. 356) determined by calipers, and recorded in centimeters. The stomach was then rapidly removed, the subdiphragmatic oesophagus and also a small bit of the duodenum having been left attached in the earlier ones for orientation. The stomach was then slit open along either anterior or posterior side, and filled with fixing fluid. Stomachs from embryos of over 6 cm. length are distended with a clear, glairy, mucoid fluid, often of a greenish tinge. This must first be allowed to escape, as otherwise fixation is unsatisfactory. After being filled with fixing fluid, the stomach is immersed in it and placed in the dark. After a little practice the whole operation need take no longer than half a minute. This fixation immediately after death is of great importance in obtaining accurate pictures of intracellular conditions, especially of the zymogenic, mucus and parietal cell granules. In dealing with embryos of over 12 cm. some of the stomachs were treated as described above, others were subdivided, great care being taken to preserve the identity and orientation of the pieces. The series included embryos of 2 to 29 cm. length at intervals of $\frac{1}{2}$ cm. Generally, several stomachs of each stage were used; some for sagittal sections, thro' the whole length where practicable, some for cross sections. In all cases the sections were cut serially, and this is very necessary, as will be seen later, for the correct interpretation of certain appearances.

I tried many methods of fixation, but finally settled down to the use of Bensley's fluid as giving the best general results.[1] Modified

[1] This consists of equal parts of 3 per cent aqueous K2Cr207 and sat. HgC12 in 100 per cent alcohol, mixed just before using. The cloudy precipitate is to be disregarded. The material is fixed in the dark from 30 minutes to 2 hours, according to thickness. It is then transferred to 50 per cent alcohol, in which, with frequent changes, it remains several days; then to 70, 80 and 95 per cent a day or two, with several changes in each; finally to 100 per cent and then bergamot, bergamot and paraffine, and finally paraffine. At no time until after impregnation with paraffine should the material be subjected to the action of water. Otherwise, as Bensley pointed out (1903-4) the zymogen granules and mucigen are preserved poorly or not at all.

Köpsch is a good fixative for Bensley's three color stain (see below). Controls were often made with Zenker and 5 per cent freshly distilled formalin, but these only served to emphasize the superiority of Bensley's fluid, especially for the neutral gentian and copper chrome stains. The eosinophile granules of the parietals, the zymogenic granules and the contents of mucigenous cells are especially well preserved by this fluid.

Sections were cut $\frac{1}{3}$ μ thick, and fixed to slide by the water method, or by Mayer's albumin. Demercurization was completed by immersion of slides in iodine-alcohol.

Hematoxylin and eosin stains were used in each stage for comparison, but the main reliance was placed upon the four stains named below, serial sections of each stage being stained with each of these:

1. Bensley's three color stain as described by Klein ('06) p. 323. The granules of parietal cells are stained a vivid cherry-red and the intercellular ductules are brought out well. This has proved the best all around stain for stomach material.

2. Neutral gentian (Bensley '00) in 20 per cent alcoholic solution was invaluable in determining the appearance of zymogenic granules. It is also, as far as my experience goes, the best cement line stain, and is thus of great value in the study of the parietal ductules, and of cell boundaries. The material should never be brought into water, as the zymogen granules then disappear. I stained two to four days.

3. Bensley's Copper chrome hematoxylin as described by Harvey ('07, p. 209). The granules of the parietals are stained a steel blue or deep blue black. The chromatin stains black, but is more readily destained than are the parietal granules.

4. Mayer's Muchematin, as modified by Bensley ('03, p. 11) for the detection of mucigen. It is best to avoid the water and heat method in fastening sections to slide, as the granular form of the mucigen is thereby altered.

HISTOGENESIS OF THE GASTRIC GLANDS OF THE PIG

1. The early stages

The stomach of a 2 cm. pig is about 2 mm. long. The cephalad part just left of the oesophagus has already been partially folded off as the secondary cardiac pouch (Coecum) being now separated from the main lumen by a fold which isolates it.[2] This ridge is an infolding of all the layers, including the tunica muscularis, the latter and the connective tissue coat being also thickened at this point.

A definite basement membrane separates epithelium from mesoblastic coats. This stains densely with Rubin S, and has a fibrous structure.

The mesoblast has already differentiated into the connective tissue layer, the tunica muscularis and the serosa. Only the first concerns us. It is a typical mesenchyme, with numerous stellate and spindle cells, anastomosing by their processes. The nuclei are spherical, ovoid or elongated and many are in mitosis. The ground substance is transparent and gelatinous. Already this connective tissue coat has definitely differentiated into two strata (fig. 1).

The cells and nuclei of the inner layer, just beneath the basement membrane, are very closely set, almost touching. Mitoses are very numerous. There is here but little of the mucoid, intercellular matrix, and it is dense, staining somewhat reddish or purplish with the three color or H & E. The blood vessels are of capillary size only. The endothelial cells of the vessel walls are very frequently mitotic. This layer is the young lamina propria mucosae.

Outside this is a broader zone with cells and nuclei sparsely distributed, and a corresponding predominance of intercellular substance, the latter staining hardly at all, and being very clear and transparent. Mitoses occur, but are rare. Blood vessels are very numerous and many of them are of large size. This coat

[2] For the topography of the adult stomach, reference may be made to Greenwood's accurate diagram, Fig. 252, Oppel, 1896.

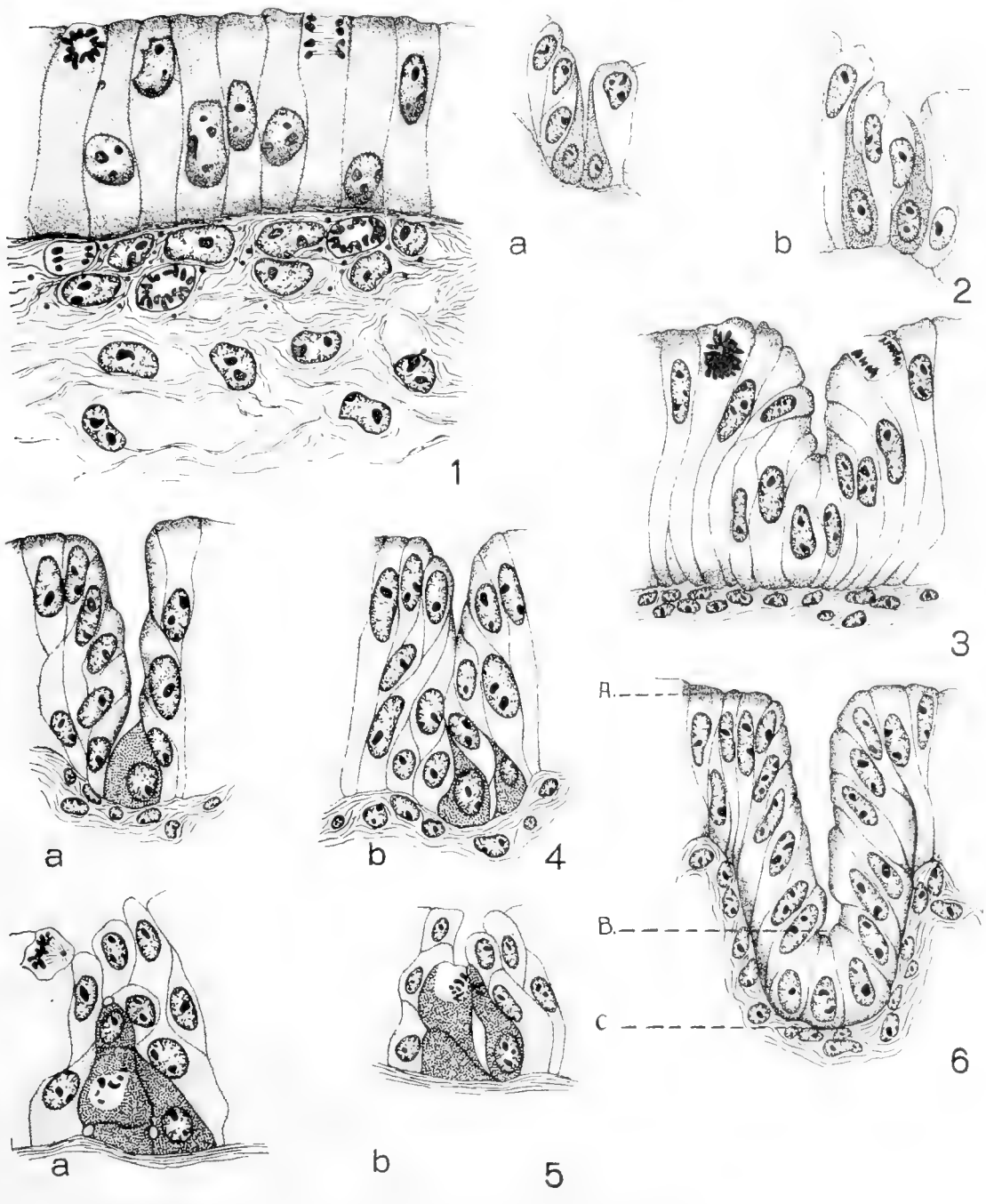


Fig. 1. Earliest or preglandular stage. Epithelium and underlying mesoblast 6 cm. embryo. Three color stain.

Fig. 2. Young parietal cells, showing intercellular ductules. 6 cm. Fundus. Three color.

Fig. 3. Intra-epithelial gland stage. 6 cm. fundus. Three color.

Figs. 4 and 5. Young glands cut obliquely. 6 cm. fundus. Three color. 5a and 5b represent adjacent sections; similarly 6a and 6b. These illustrate the true nature of the interbasal groups of Toldt and Ross.

Fig. 6. Fundus gland. 6 cm. parietal cells were present in other sections of this same tubule. Three color.

is the young tela submucosa. The boundary between these two layers, while not absolutely sharp, is quite well marked, being definable within the limit of one or two cells breadths.

There is yet no trace of a muscularis mucosae, this first appearing about 9 or 10 cm. at the boundary between lamina propria and tela submucosa. It originates through the elongation of mesenchyme nuclei to a torpedo shape, and the condensation of the protoplasm about each nucleus, as a highly eosinophile substance. The primitive syncitial anastomoses are retained, the new muscle cells being thus joined *inter se* by delicate prolongations of the finely fibrous stroma.[3] In some stomachs isolated muscle fibres apparently appear somewhat earlier (8-½, 9 cm.) At 15-16 cm. a definite, fairly compact muscularis mucosae is present. The fibres of both coats of the tunica muscularis are well defined at 2 cm.

The diversity of opinion as to the part taken in the inception of the glands by unequal mesodermic growth, and as to the nature of the first mesodermic irregularities, is partially referable, no doubt, to differences in the forms used, but there has often been the lack of a definite criterion of distinction between such upgrowths as simply minister to an increase of surface, and such as have a definite rôle in glandular development. By reference to the adult structure, it is seen that *only such mesodermic elevations can have taken an active part in adenogenesis as are constituted by local thickenings of the lamina propria alone.* At 2 cm. the later is of uniform thickness throughout, the basement membrane presenting no irregularities of contour, and the epithelium being everywhere of the same height. But the tela submucosa has thickened in linear ridges, so as to produce folds or rugae, several of which run almost the length of the stomach.

The epithelium is a single layer of high columnar cells, as shown in fig. 1 of the 6 cm. stage The nuclei are arranged in several rows; this, in connection with the small diameter of the cells, readily gives rise, in even slightly oblique sections, to a stratified

[3] This confirms McGill, 1907.

appearance. At no stage does the epithelium acquire stratification except in the pars oesophagea.[4]

The nuclei really occur at all heights in the cells, but, may, for convenience, be arbitrarily grouped into those occupying the distal middle and basal parts. At 2-3 cm. almost every nucleus of the distal row is in mitosis, and all stages of this process are, of course, represented. The nuclei lying at deeper levels are all resting. Whenever the mitosis is in such a stage that its direction may be determined, its axis is invariably parallel to the surface of the epithelium. The nuclei are oval, saccular, and so broad as to almost touch the sides of the cell. They have a moderate amount of chromatin, arranged as a net, and with nodal karyosomes. There are usually two nucleoli, metachromatic or slightly acidophile. The cytoplasm is very finely granular in the epithelium of the coecum, cardia and cardiac part of the fundus; in the plyorus, pyloric part of the fundus and pars oesophagea it is clear and transparent. Aside from this, no cyto-differentiation is discoverable by the techniques employed.

At 2½ cm. irregular thickenings of the tela submucosa in the coecal pouch begin to give rise to the characteristic grosser ridges folds and papillae of that region.

About 2½-3 cm. the surface line of the epithelium, hitherto level, becomes, in some parts of the stomach (pylorus and fundus along the greater curve near the pyloric region), undulatory, displaying alternate very slight elevations and depressions. The basement membrane shows no corresponding waviness, and the lamina prop. no irregularities. On reconstruction, the elevations are found to be short ridges, intersecting in all directions, the depressions thus representing a slight pit bounded on all sides by the ridges. It is readily determined that the elevations consist of cells slightly taller and narrower than those of the depressions (fig. 3), with the nuclei in the distal end; the cells of the depressions have central or basal nuclei, and have retained their primitive height and breadth, or, sometimes increased slightly in breadth. Mitoses are very frequent in the ridge cells. *All the cells reach from basement membrane to surface.* These slight intraepi-

[4] This finding agrees with those of all the later investigators, as Toldt, Salvioli, Ross.

thelial depressions, due to irregularity in height of the epithelial cells, represent the beginnings of the glands, as I have satisfied myself by tracing, in unbroken series, all steps from the adult glands back to these. It will be convenient, hereafter, to refer to this as the *intra-epithelial stage of* gland formation.

Nevertheless, it is hardly possible to say that the mesoderm plays no part in even the initial stages, for these epithelial ridges have scarcely appeared before mesodermic buds begin to push up into them (fig. 4). These latter are constituted by local thickenings involving only the lam. propria, and are true mesodermic gland processes.[5] This latter condition is found rarely at 2½ cm. but often at 3 cm. in the precocious regions. As soon as mesodermic cores appear, the basement membrane is seen to be sharper in outline, and more compact, beneath the pit or gland cells than beneath the ridge cells, being frayed out in the latter locality into fibres which penetrate beween the bases of the cells. Fig. 6, indicates this same condition in a later stage. Possibly this has some influence on the supply of nutrition to the epithelium, and consequently on local differentiation of the latter(?).

The process just described is, in essence, the same as Salvioli's finding in the rabbit, but there are minor differences:

1. In the pig the cells of elevation and depression do not diverge quite so widely in form, in these earliest stages. But at the stage represented in fig. 3 the form divergence seems quite as great.

2. In the pig, there is yet no differentiation, as to the cytoplasm into two types of cells, as Salvioli's clear cells of the elevations, and granular cells of the depressions. Instead all the cells in the areas of gland formation, possess, as yet, a finely granular, slightly acidophile cytoplasm. The cells of the fundus and pylorus were non-granular at 2 cm. but, shortly before the appearance of the intra-epithelial gland anlagen, have acquired the fine cytoplasmic granulation.

[5] This term of Sewall has the priority. His gland processes undoubtedly correspond to these, and despite Ross' criticism, certainly play an integral part in gland development.

3. In the pig, the mesodermic cores appear relatively much earlier—in fact so early that it was only after careful reconstructions that the appearance of epithelial ridges slightly in advance was established. Toldt's description for the pylorus is very similar. He admits that in this region there is no independent origin of the gland cells, with, later, secondary communications between these and the lumen.

From 3 cm. on, the ridges of lamina propria grow rapidly up into the epithelial ridges (figs. 4 and 6), causing a correspondingly rapid heightening of the latter, and deepening of the glands. For the correct interpretation of certain papilloid structures seen in all stages of the gastric development up to about 23-27 cm., it is essential to understand the exact way in which the irregularity of mesoblastic growth manifests itself. As has been indicated, the early, intra-epithelial ridges intersect in all directions. They are not straight, nor do they intersect often at right angles; rather they are short, very irregular, curved or angular, and they intersect at all angles. Now, when the mesoblastic ridges begin to reinforce them from below, the mesoblast pushes up much more rapidly at the nodal points of intersection of the ridges than along the intervening parts. The result is that in a sagittal section taken (fig. 7A) through the line a, b, c, d, the appearance seen in fig. 7B results. That is, we have in addition to the ridges the papilloid elevations a, c, and d, situated at the intersections of the ridges, and higher than the rest of the interglandular ridge. In cross or oblique section of the upper level of the mucosa, they are, of course very numerous, and appear like true papillae. One might readily gain the impressions, as did Brandt and others, that in the early stages there are no gland processes, but simply papillae, the depressed areas between them communicating with each other. Such a condition would be represented by fig. 7A, with all elevations except the nodal points effaced. But this is very different from the condition actually found—namely, that ridges, not so high, it is true, as the papillae, but still high in comparison with the depressed areas, run across from the base of each papilla to the adjacent one. This papilloid condition is marked from the

time of the appearance of the mesoblastic reinforcements to the 20-26. cm. stage, but all this time there is a very gradual increase in height of intervening ridges, until finally at 24–28 cm. the definitive condition is reached, in which there are no longer any papilliform elevations, all the ridges having grown up to the same height as the papillae.

The nodal papillae are often expanded or club-shaped at the top, resembling greatly intestinal villi, but of course shorter. By reference to fig. 7B, it will be seen that in any section through the mucosa the elevations and depressions must necessarily present great irregularities, as *e. g.*, the epithelial line may dip into a gland pit, then perhaps ascend to the top of a nodal elevation, then descend to the level of an interglandular ridge.

Moreover, these ridges and pits cannot be interpreted as mere folds of the mucosa, having no permanent significance, nor any relationship to the adult gland. Once established, these pits indeed deepen and compound at the bottom, many of them even divide into two glands; but their continuity with the adult gland is established by unbroken series. Moreover, we shall see that soon after this formation, certain cells in their depths differentiate into recognizable elements of the adult gland. Finally, the mesoblastic cores of the ridges are, as shown, true thickenings of the lamina propria, not mere foldings of a l. p. of uniform thickness.

It is now easy to understand the many contradictory accounts of the nature of the primary gland processes. Brandt described papillæ or villæ, which later fuse at their bases. Sewall, Salvioli and many others described only the interlacing ridges, and failed to explain the very frequent appearance of villoid cross sections.

As growth proceeds, all the epithelial cells increase in absolute size. Those on the ridges become very high, inverted pyramidal in shape, the proximal part being compressed into a narrow, caudal process, which attaches to the basement membrane (figs. 6 and 11). The nuclei are elongated and laterally compressed. The cells in the pits, or embryonic gland cells[6] increase in breadth, but

[6] I retain Sewall's term, as it has priority, and is self-explanatory. Toldt calls these "adelomorphs," but applies this term at a later stage to the mucus and serous chief cells.

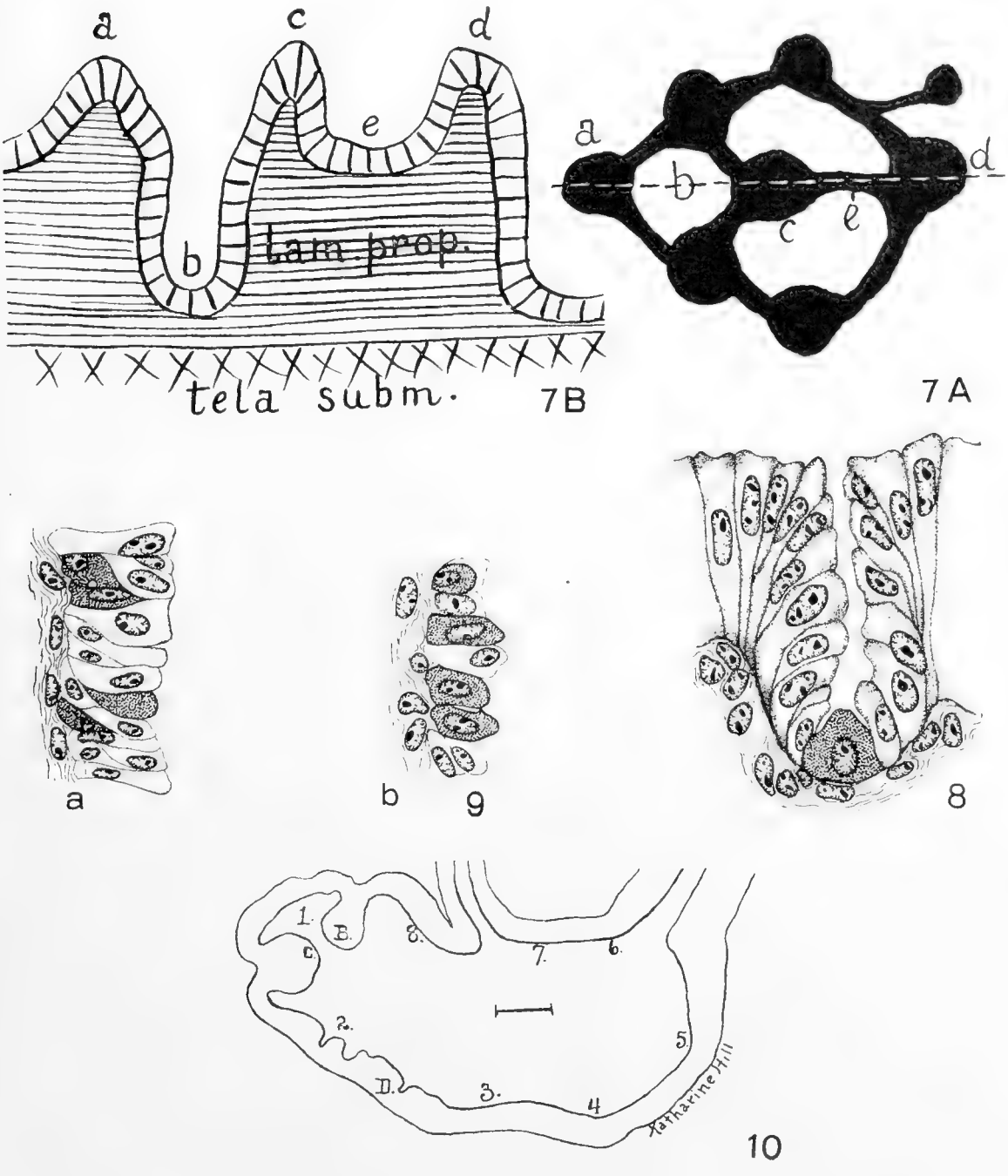


Fig. 7. A and B. Diagrams (vide text).

Fig. 8. Fundus gland. 7 cm., showing one parietal cell with ductules. The other cells are "Embryonic gland cells," *i.e.*, undifferentiated; at least they present none of the characteristic staining-reactions of any of the adult cell-types.

Fig. 9. a and b. Fundus epithelium 7 cm. Copper chrome hematoxylin.

Fig. 10. Tracing of mesial sagittal section through 7 cm. stomach enlarged x 8.6. B to C, Caecal pouch. C to 4, Cardia and fundus. 4 to pyloric aperture, and latter to 7, pyloric gland area. 7 to B, pars oesophagea. B, Caecal "Wulst" or ridge.

not in height, so that they become thick, short and cylindric, or pyramidal, with broad, basal ends. The nuclei are ovoid, or often almost spherical and moderately chromatic. In glands of the stage indicated in figs. 6 and 11, the gland cells average 16μ in length, 8μ breadth; the ridge cells, 32μ height, 3.2μ breadth at middle, 6.4μ breadth at the broadest part or distal end. Up to the stage of fig. 3 the only distinction between the epithelial cells of the pits and ridges, are those just described, of size and of contour of cell and nucleus. The cytoplasm of all these cells is quite refractile owing to the presence of very fine, slightly acidophile, granules. Up to this stage, then, we have found two factors coöperating in the deepening of the primitive gland tubules. (1) Upgrowth of the mesodermic gland processes, (2) increased height of the ridge epithelial cells, which are now over twice as high as the gland cells.

But as the mesoblastic buds reinforce the epithelial gland processes mitoses become relatively fewer in the ridge cells, and appear in the pit cells, and it soon becomes apparent that the gland tubules elongate by interstitial growth, i. e., by mitoses of the gland cells. Thus the epithelial part keeps pace with the growth of the lamina propia.

The chronological priority, however slight, of the epithelial ridges to the mesoblastic cores, seems, to indicate that, from the first, certain epithelial cells possess an inherently different potentiality from others. For the primary ridges and depressions are so minute that it is inconceivable that differences in the distribution of vascular supply in the underlying mesoblast could account for this appearance. But, waiving this, at present, fruitless discussion of the predetermination of the cells, it is obvious that the grosser differences in the external form of the ridge and pit-cells are largely a function (in the mathematical sense) of the formation of ridges and pits, i. e.,—the inverted pyriform contour of the ridge cells is largely the mechanical resultant of the compressive forces exerted at their base end and of tensile force exerted at their distal end; and the short, pyramidal form of the gland cells, of compressive forces exerted at their distal end, and of tensile force applied at the base. And these

compressive and tensile forces are obviously referable, in great measure to the uneven mesoblastic growth.

However, we shall see below that other differentiative forces, intrinsic to the cell, and of a metabolic nature, are at work, as is indicated by the early departure of certain of the gland cells from the primitive embryonic type.

These glands appear earliest in the following localities:

(1) The pyloric portion of the greater curve; (2) the part of the fundus adjacent to (1); (3) the caecal houch; (4) a narrow, peripheral zone of the pars oesophagea. There is some doubt as to whether these latter become true glands.

From these primary regions they extend to all other regions, with the exception of the major part of the pars oesophagea, i.e., that epithelial territory which later becomes stratified squamous. Thus, gland development is most retarded in the cardiac end, over the facies anterior and posterior, and especially over the caecal ridge. Moreover, apart from this general, regional precocity or retardation, several slightly different stages of development may be represented in each region.

At 4–5 cm. the pyloric glands are mostly in the stage of fig. 4 the processes having cores of mesoblast. Some are advanced almost to the stage of fig. 6. Those of the fundus are of the stage of fig. 3, while the cardia displays a slightly undulating epithelium, with numerous mitoses in the upper row of nuclei of the elevations. There is, as yet, no sharp boundary between fundus and pylorus, as to size and form of the glands, but we shall see below that already through cyto-differentiation, these two regions are marked off from each other.

Between cardia and fundus, no definite boundary of any sort is discoverable, since the change from the shallower (younger) glands of the cardia to the deeper (older) of the fundus is very gradual.

The developmental retardation, as intimated above, finds its extreme expression over the coecal ridge, where the epithelium is at this stage, level, with very numerous mitoses in the distal row of nuclei.

All the epithelial cells, both ridge and gland, with the exception of those of the pars oesophagea, present at the first appearance of the glands, the same type of cytoplasm,—highly refractile from the inclusion of a very fine, closely set, slightly acidophile granulation. These embryonic gland cells do not all remain undifferentiated very long after the appearance of the gland anlagen. At 3 cm. some of the glands of the stage of fig. 3 or even slightly earlier, situated in the fundus, along the greater curve, display one or two cells, which have acquired a cytoplasmic granulation somewhat coarser than that of the primitive cells, and very highly eosinophile. These cells are at first of the same size and contour as the others (figs. 4 and 9) but they soon enlarge somewhat, the basal end becoming broader and rounded, while the distal end narrows, often becoming caudate (fig. 2). Later, the narrow distal end is lost (retracted?), but at all times, up to 24 cm. the cells reach the surface (fig. 8, etc.). The nucleus, at first ovoid, later rounds up, and approaches the spheroid form. These cells are the earliest parietals. Even at their first appearance, one often finds, in relation to them, minute intercellular ductules, always continuous with the lumen of the gland, or, in the more shallow glands, opening directly into the stomach lumen (figs. 2, 4, 5). These pass down between the parietal and the adjacent primitive cells. The almost absolute constancy with which these early parietals display the ductules is striking. As already intimated, parietals sometimes appear in insinkings so slight that they seem almost to have differentiated in level epithelium. This occasionally occurs as late as 7–8 cm. (fig. 9) in small patches of epithelium which remain almost level in the midst of active gland formation on all sides, as also in the extreme cardia at a stage when the glands are just appearing. Nevertheless, even these parietals are probably always situated within the territory of a gland-to-be, for after the 10 cm. stage, parietals are found only in bona fide glands, and no primary gland-pits appear later than this stage. Transitional forms between embryonic gland cells and parietals are also found, their cytoplasmic granules being intermediate in size and avidity for acid stain. These occur not only in the early stages, but even

at 11–12 cm. and probably up to 19 cm. A point which I wish to emphasize is that the glands are largely constituted from the very first, of adelomorphs.[7] Some glands have parietals almost from the first; some do not develop any for some time. Thus, as late as 6–7 cm. glands occur, especially in the cardia, but also in the fundus, in which all the cells are undifferentiated. And by the side of these will be found glands of the same age (size) but with one or more parietals. I am able to confirm Toldt's statement that parietals do not appear in the pylorus at any stage. However, once or twice,—so very rarely that even to mention it is to give an exaggerated idea of the importance,—I have found a single parietal in a young gland from the undoubted regio pylorica; never, however, a group of parietals. Also, while in later stages, the boundary between fundus and pylorus is very sharp on the greater curve, there is, at some points on the facies anterior and posterior a zone, two or three tubules wide, in which occur mucus chief and parietal cells, but no zymogenics.

Thus, with the differentiation of parietals, we have at once a rough divergence of left gastric region, corresponding to caecal pouch, cardia and fundus, where they appear, and right gastric region, the pylorus, where they never appear. Merely a rough divergence at first, because, in the earliest stages, not all fundic tubules have parietals. Thus the parietals arise at first, by the differentiation of certain of the adelomorphs; but, as early as 4–5 cm. they increase also by mitosis (fig. 5). This method of division in parietals has been denied or doubted by many observers, but I can say positively that it frequently occurs up to rather late stages of glandular development, certainly as late as 16 cm. The eosinophile granules remain intact, but a clear zone, free from them, surrounds the chromatic figure. As soon as groups of 2 or 3 have thus arisen the ductules are formed not only between the parietals and adjacent embryonic cells, but also between adjacent parietals (Figs. 2, 4, 5). Thus, by mitotic division of preëxisting ones, and by differentiation of new ones

[7] This convenient term, used by Toldt in the same connection, may, for brevity, be used interchangeably with primitive gland cell, care being taken not to apply it, as did Toldt, to the specialized mucous and serous chief cells.

from adelomorphs, larger and larger groups and rows appear in later stages. I do not believe that new ones develop from adelomorphs after 13–14 cm. and it seems almost certain that this does not occur after 19–20 cm., as all the adelomorphs have at that time differentiated into other types, mucus or zymogenic. Thus, in the latest stages, the parietals probably arise only by division of parietal predecessors, unless there is a genetic relation between parietals and zymogenic or mucus cells, of which I found no evidences.

If the section of the early gland be even slightly oblique the parietals, whether isolated or in small groups, appear as conspicuous, large, granular cells, lying apparently between the bases of the surrounding adelomorphs and often as if far removed from the surface. If cut obliquely, the cells appear of ovoid contour, if cut transversely, of circular outline, hence sometimes interpreted as referable to a spherical shape (Ross, '03). Figs. 4b, 5a, and 9a illustrate this condition, as do Ross' ('03) figs. 25, 26, and 24. *By reconstruction it is found that they reach the surface. Vide* figs. 4a representing the section adjacent to 4b, 5b adjacent to 5a; 9b where obliquely cut parietals are shown, including the distal end of one. These obliquely cut parietals are much more conspicuous objects than are the obliquely cut bases of the surrounding adelomorphs, owing to their larger size, granulation and deep red color. This probably accounts for the inconsistency of the interpretations of Ross, whose figures show the obliquely cut bases of parietal cells surrounded on all sides by undifferentiated cells, likewise cut obliquely. The parietal cell bases are then described as basal gland anlagen, which have never yet approached the surface of the epithelium; curiously, this interpretation is not extended to the obliquely cut bases of the adelomorphs. Ross' figs. 28 and 29 are especially significant as showing the true condition, and are in perfect accord with my findings. The little depressions mentioned by Toldt and Ross (Ross '03, fig. 24d) and interpreted as surface insinkings which later communicate with the fundus of the gland, are shown in my fig. 4b etc., but a glance at adjacent sections (as 4a) always shows that they are merely part of the shallow lumen of the gland,

i. e., have been from the first in communication with the lumen of the gland fundus. Toldt and Ross have both spoken of the appearance of a minute vacuole between the cells of a group; thus Ross: "A central lumen, small, to be sure, but still a lumen" (fig. 26). This is said to have arisen independently within the group, and to have secondarily communicated with the surface. They figure and describe very accurately the appearance of the intercellular parietal ductules, as seen in cross or oblique sections (*Vide* my figs. 2, 4, 5).

Salvioli, too, ('90) has pointed out the source of Toldt's error ('81)—namely, oblique sections, with no reconstruction control—but this warning was evidently overlooked or disregarded by Ross ('03). Ross pictures similar groups of basal cells for Amblystoma and the pig but the two are different in nature. The basal group figured for Amblystoma are undoubtedly cells of the fundus segment of the glands the latter possessing but one type of cell,—namely, those which become, in the adult, zymogen cells. But Ross homologises with these groups, those of somewhat similar appearance in the pig; and the latter are, as I have shown,—simply certain very conspicuous ones of the fundic segment cells,—namely, the newly differentiated parietals.

How may we know that these are really young parietals? Because they can be traced, in unbroken lineage, from the 3 cm. stage to the stage just before birth (29 cm.), long before which they are definitive in size, position and all morphologic characters; because from their first appearance, they exhibit the slight enlargement as compared with the adelomorphs, the conspicuous intercellular ductules and the cytoplasmic granules, the latter, at all stages, showing a characteristic affinity for Rubin S and Eosin, and staining black or steel blue with copper chrome hematoxylin, and copper red with neutral gentian. Certain other characteristics are not so marked until the later stages; such are the polygonal, spherical or lenticular shape, which succeeds the earlier piriform shape. The nucleus of the early parietal is ovoid, as are those of all the gland cells, but by 6 cm. many of the older parietals have spheroidal nuclei. This latter, however, is not a constant character, as, even in the adult, parietals are found with ovoid nuclei.

We have seen, then, that all the cells of the gland tubule, are, from the first, on the same morphologic level; all reach the surface and the basement membrane in the early stages; there are no "basal cells" in the sense of cells which are shut off from the stomach lumen by higher cells. Moreover, the parietals are the first of the adult gastric cell-types to be differentiated. We shall find that, in the earlier stages,[8] they appear only in the lower parts of the gland tubule.

While the epithelium of all other parts of the stomach has become granular and refractile, that of the pars oesophagea has remained absolutely clear and transparent. As had been anticipated, no glands appear in the major portion of this territory, which at 4 cm. yet presents a simple columnar epithelium. But a small peripheral one has already developed glands, by the same general process described for other regions. Their cells, like those of the rest of the pars oesophagea, are absolutely clear, and the distinction from adjacent regions is very sharp, so abrupt that one is able to compare the last transparent cell with the adjacent finely granular cell of cardia, fundus or pylorus.

The caecal ridge is covered by simple, granular epithelium which displays the slight waviness and the numerous distal mitoses seen in the 2 cm. stage of the fundus and cardia.

In the caecal pouch, development is exceedingly erratic. At 2–3 cm. slight irregularities occur; some of these elevations are broad and being due to unequal growth of the submucosa, correspond, when linear, to the gross rugae, and, when circumscribed, to the gross papillae or villi, of the adult caecum; others are due to a thickening of the lamina propria alone, but are very broad, or very high, and manifestly have no direct relation to gland formation, helping to increase the surface area. Other stretches are yet level and present numerous mitoses in the distal row of nuclei. At other places, the first, minute, intraepithelial glandular depressions are appearing. At 4 cm. some of these glands are in the stage of fig. 3, some of fig. 6. They are rather sparsely distributed. Thus, the small caecal pouch shows a developmental

[8] Thus up to about 7 cm., in the fundus, and about 12 cm. in the cardia.

picture as varied as that of all other parts of the stomach together, including within its limits all types of glandular development found at these stages in the stomach. It is thus defined by its gross boundaries, rather than by any well marked histologic or glandular type. By 7 cm. the rugae and papillae have by the successive appearance of smaller ones on the larger, acquired complex, fantastical shapes, like those seen in a papilloma. The comparatively few glands appear both on these rugae and in the depressions between them. Some of them have progressed to the stages of Figs. 6 and 11, and hence resemble the pyloric glands in size and form, but have developed some parietal cells.

Mucus cells appear, for the first, in the 6–6.5 cm. stomach, at which time all the cells in the lesser curve (pyloric) area stain very definitely and deeply with muchematin. In general, the surface cells and those near the top of the gland have but a shallow, distal rim of mucus, the rest of the cytoplasm being of the ordinary, granular type. In the deep cells of the tubules, the cytoplasm distal to the ovoid nucleus is of alveolar structure, the alveoli being filled with mucin. If care is taken to preserve the tissue from the action of water, the mucus occurs in the form of spherules, one in each alveolus (fig. 12), and enclosed by the slender alveolar wall. With the three color, or hematoxylin and eosin stain, the cytoplasmic part of these cells, surrounding the nucleus, and in the first mentioned type, occupying the whole cell except the distal rim, presents the ordinary, finely granular, slightly reddish appearance of the ordinary embryonic cells; the mucus part appears clear, and has an alveolar network. In the other parts of the pylorus (greater curve and facies anterior and posterior) no mucus cells have appeared, all the cells being of the primitive type. As to size and form, the glands are uniform in all parts of the pylorus.

At 7 cm. the region from C to 4 (fig. 10) represents the territory of the adult cardia and fundus. There is a gradual and progressive increase from C to 4 in the age of the glands. Those toward C are in the intraepithelial stage (fig. 3). They occur clear up to the caecal ridge, where the epithelium becomes level. Toward 4 the glands approach the type of fig. 7, but are

not quite so deep, the mesoblastic cores of the processes being smaller. Many glands in the whole area C to 4 possess parietals, which in the part toward C are isolated, groups being often found toward 4. Thus cardia and fundus merge insensibly as yet, the fundus being as always, more precocious. The fundic glands, from about D to 4, present at 7 cm., the following measurements: The lumen depth[9] averages 25μ; the process height, 35–40μ. Mitoses now occur in the tubules, mostly about one quarter the distance from the surface, i.e., in the region corresponding to the adult neck.

At point 4 an abrupt change occurs, the glands from 4 to the pyloric aperture being almost twice as deep as the fundic glands, the average lumen depth being 40–50μ, the process height, 50–70μ. The gland processes are broader (32–40μ) than in the fundic glands. These pyloric glands also occupy the lesser curve, from the pylorus to point 7, where the pars oesophagea abruptly begins. The pyloric glands and processes are strikingly symmetrical, the former having the form of simple tubes. Many mitoses are present at all depths, not so many on the surface.

It must not be forgotten that in the cardia, fundus and pylorus longitudinal rugae occur, some running the whole length of the stomach. They are not so large as those of the caecum, but are readily visible with a hand lens. In the cardia, they are especially large ($\frac{1}{8}$–$\frac{1}{4}$ mm. in diameter), and often papilliform or highly irregular. The caecal ridges are very high.

Parietals now occur in caecum, cardia and fundus; in the latter, groups of them. We have seen that the first to appear, came to occupy only the deeper parts of the tubule. But now, in the deeper tubules (from 3 to 4, fig. 10) new ones are differentiated farther up along the sides, at first as single cells, which, by mitosis, soon give rise to groups in this new position. They never appear in the upper quarter of the tubule.

At 7 cm. the mucous cells have spread down to point 5 on the greater curve, the pyloric glands from 5 to 4, yet containing no trace of mucin. The boundary between the mucous and non-

[9] For brevity the distance, fig. 6, from A to B will be called "lumen depth;" from A to C "process height."

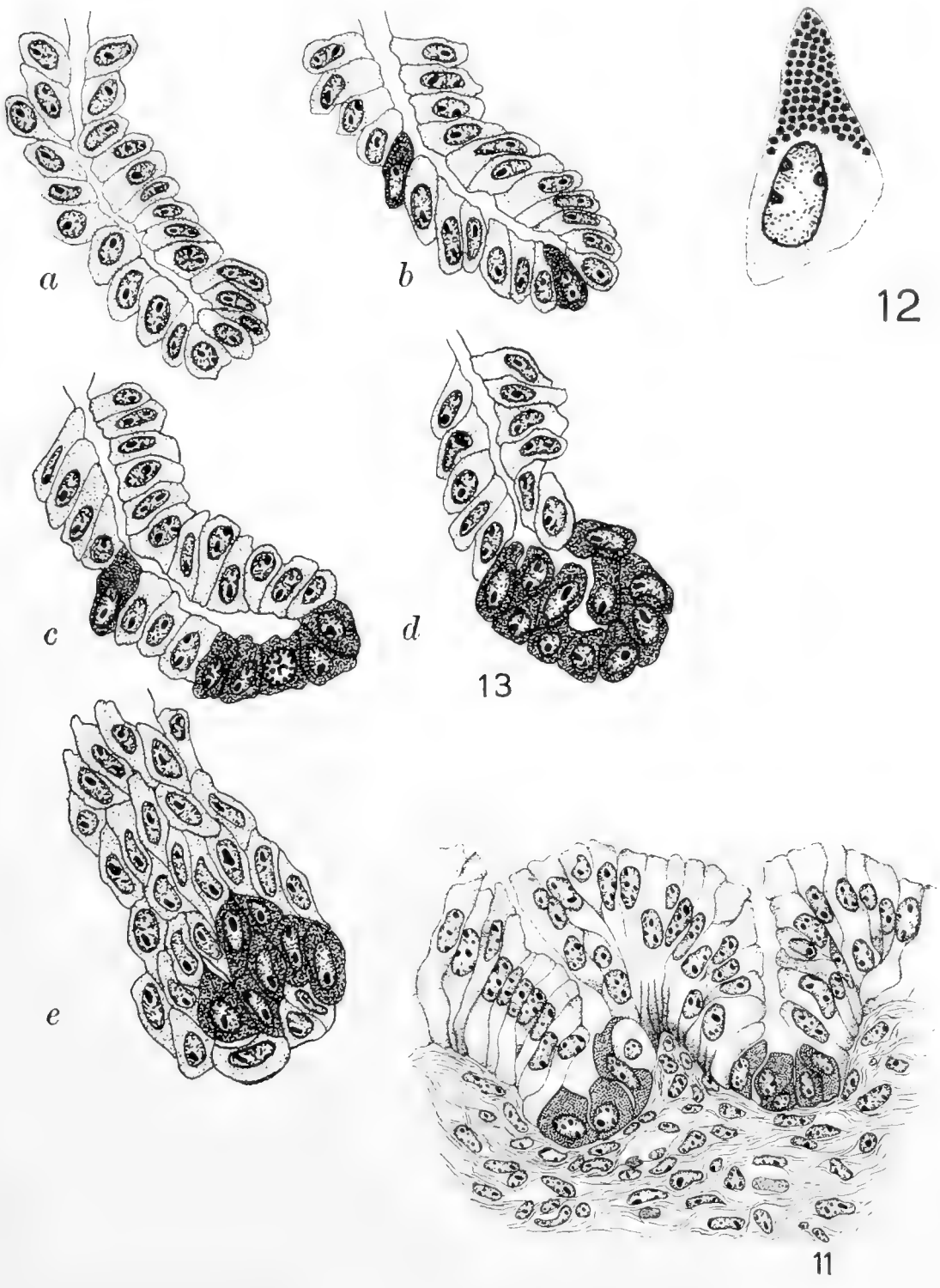


Fig. 11. Tubules of cardia near fundus. 7 cm. Three color.

Fig. 12. Mucous chief cell from bottom of a pyloric tubule. 10 cm. Muchaematin.

Fig. 13. Adjacent serial sections through a tubule of fundus region. 10 cm. Three color.

mucous pyloric tubules is absolutely sharp. Many of the mucous cells in the gland tubules are mitotic, as are also the embryonic cells. The transition, then, between fundus and pylorus is absolute (point 4) and marked by the sudden difference in size of tubules, and by absence of parietals in the pylorus. Rarely, the surface fundic cells show a very slight line of mucous stain, very inconstant, and probably absorbed from the general stomach content.

From now on, mitotic activity is incessant at all depths of all the gland tubules. Surface mitoses also occur, but are much rarer.

In the pylorus of 7–8 cm., and fundus of 9–10 cm., secondary gland processes appear at the bottoms of the primary tubules. They are of the same general nature as the primary processes, being intra epithelial for a short time, but being very soon reinforced by mesoblast. By this upgrowth they at first render the lower portion of the tubule compound, but as they progress until they reach the surface, the result is not a compound tubule, but two independent glands. This mode of increase of the glands is very common in fundus and pylorus, in these earlier stages. In the cardia, the same process occurs later (14–15cm.). These upgrowths are of the same nature as the primary ones, being intra-epithelial for a short time, but being speedily reinforced by mesoblast.

The true compounding occurs somewhat later, with the failure of these secondary processes to reach the surface. This begins in the pylorus about 17 cm., in the fundus about 19–20 cm., in the cardia about 21–22 cm. During this compounding, there is enormous proliferative activity in the depths of the tubules, as evinced by the numerous mitoses, and by the rapid downward elongation of the glands. This applies especially to fundus and pylorus, but many mitoses are found even in the cardia.[10]

After the gland-compounding has been largely completed (25–29 cm.), the mitoses are somewhat less frequent, but are still present in all parts of the tubule, especially in the elongating fundus segments.

[10] In the compounding of the cardiac tubules we shall find a complicating factor, the evagination of parietal tubules.

It was deemed best to deal with the stomach as a whole in the discussion of these earlier stages, up to and inclusive of 7 cm. But inasmuch as certain definite gastric regions have now differentiated, the development of these will, in the interest of clearness, be followed separately.

2. Pylorus

At 9 cm. all the pyloric epithelium, both surface and glandular, consists of mucous cells. The deeper tubule cells are of the mucous chief type, those higher in the tubule have shallow thecae, while the surface cells display a mere distal rim of mucus. No parietals are present, except in two or three tubules next to the fundic zone, and in these only a very few scattered ones. Now, as earlier the fundo-pyloric boundary is very definite. If, as seems justifiable in the light of the adult structure, our criterion of a pyloric tubule be the mucous chief cell-lining of the deep segment, then the fundo-pyloric boundary is absolutely sharp. For, if adjacent sections stained with muchematin and three color be compared, we find that the fundic segment of the fundus glands, even the last, has no mucous cells, while, the fundus of the pyloric tubules is lined exclusively by mucous chief cells, except for the scattered parietal or two in the last two or three tubules toward the fundus.

The pyloric processes are, in general, broader (40–54μ) than those of the fundus (25–40μ), but resemble the latter in often possessing expanded or clubbed tops. The pyloric tubules usually present a very symmetrical test-tube form. Their appearance at 9 cm. is indicated by fig. 21, for although the latter is taken from a much older embryo (19 cm.) yet the change in the pyloric glands, after 9 cm. are mainly those of size and of the upgrowth of the enclosing ridges rather than any fundamental alterations in form and cytology. In the pyloric tubules, as in those of the fundic region, there are from now on, many mitoses, and at all depths. They also occur in the ridge cells, although not so frequently.

At 10–11 cm., the deep cells are so packed with mucus that the nuclei are often flattened; the goblets of surface and foveola cells are also becoming deeper.

At 15 cm., many of the surface cells have goblets reaching clear to the nucleus, while some show merely a superficial rim, there being no regularity in the distribution of these types.

At 18 cm., the mucous chief cells line the lower third or quarter of the tubule, the goblets the upper $\frac{2}{3}$ or $\frac{3}{4}$, the deeper in position having, as hitherto, the shallower goblets. At 26 cm., all the surface and foveolar cells have very deep goblets.

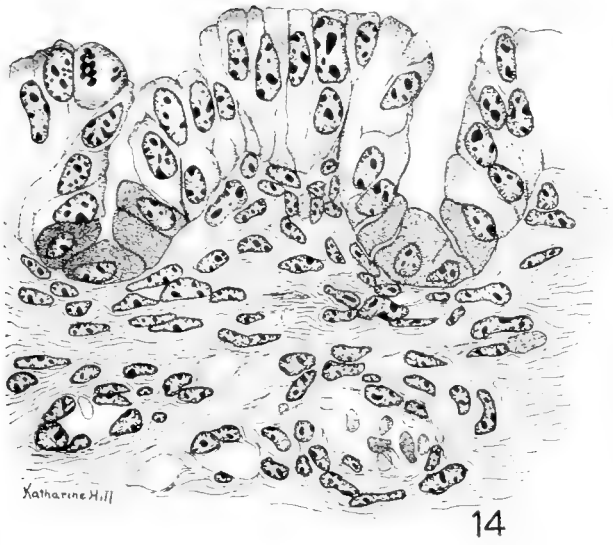
Thus cyto-differentiation in the pylorus is practically complete at 9 cm. The size of the cells, the depth of the goblets and other details alter somewhat later, but, microchemically and presumably, metabolically, the cells have then reached their definitive condition. I do not mean to imply that they have reached a terminus of potential differentiation. Harvey's work ('07) shows that very probably even the adult gastric cells, highly specialized as we are accustomed to consider them, may under changed conditions, pathologic or experimental, assume other forms and functions. But this seems not to occur in normal, embryological cyto-differentiation in the pylorus.

Measurements were taken of glands from each region at all stages but inasmuch as such figures have no general embryological value, I quote only a few, and these for the purpose of comparing the rapidity of growth in these regions. Such figures are necessarily only approximate, as there are considerable differences between those from embryos of the same length, and also between adjacent tubules.

	9cm.	16cm.	19cm	23cm.	29cm.
Cardia	35–50μ	80μ	105μ	112μ	160μ
Fundus	70μ	110μ	140μ	165μ	210μ
Pylorus	80–110μ	130μ	160μ	208μ	240μ

3. Fundus

From the first we have found considerable difference between cardiac and fundic tubules, but the two districts have always merged insensibly and the distinctions have been those of size and age, depending on the general precocity of the right and retarda-



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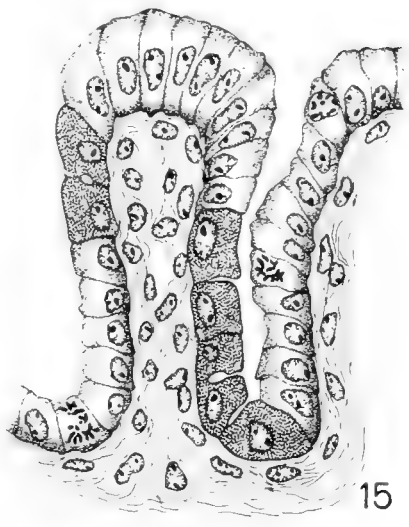

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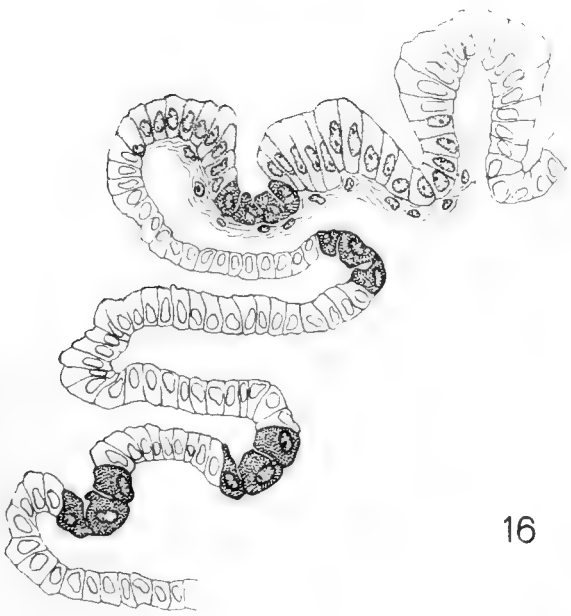

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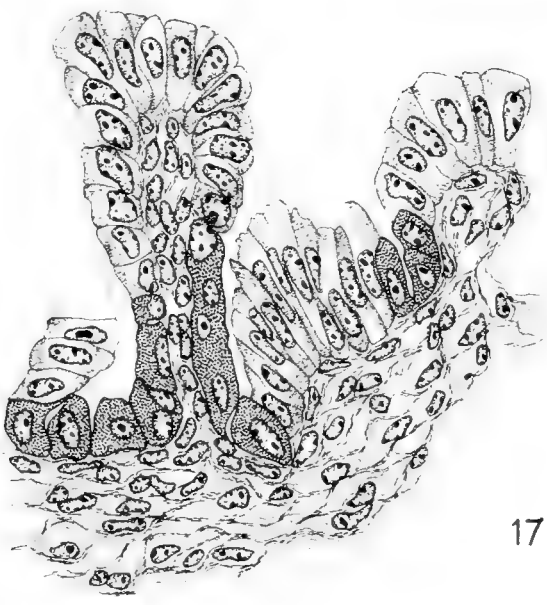

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Fig. 14. Cardia. 15 cm. Three color.

Fig. 15. Fundus tubules. 15 cm. Note ductules of the parietal cells and "lateral parietal rows." Three color.

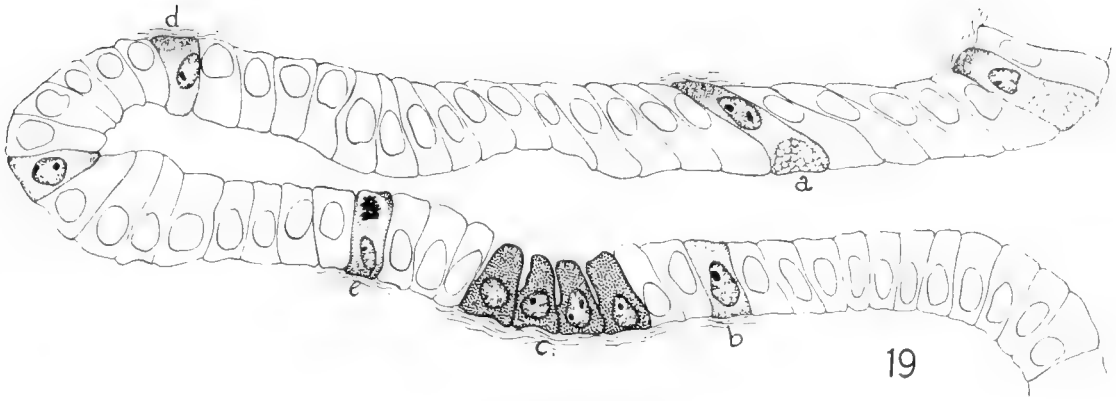
Fig. 16. Cardiac tubules. 18 cm. Three color.

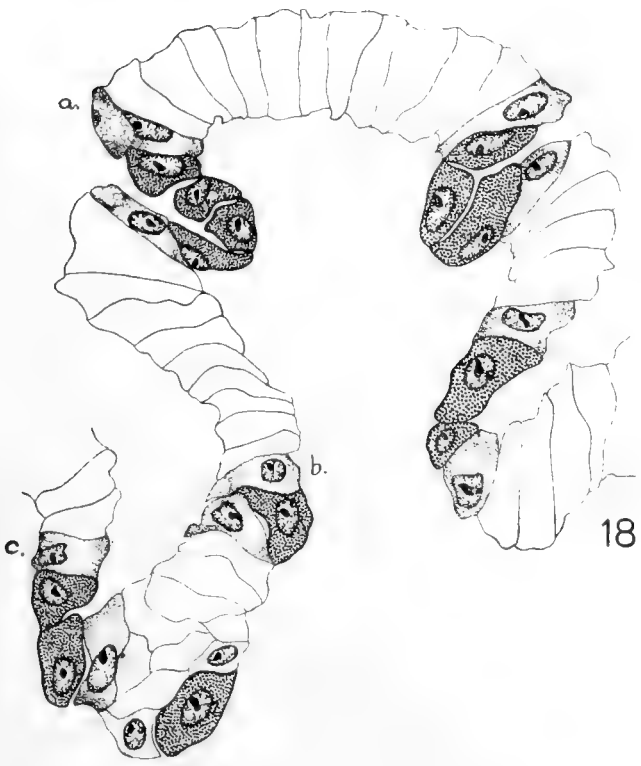
Fig. 17. Cardia. 19 cm. Three color.

tion of the left region, rather than on any true, differential divergence. Nor, indeed, shall we expect to find, at any time, a sharp boundary comparable to that between the fundus and pylorus—for such does not exist in the adult pig. (*Vide* Greenwood '85; Bensley '02, p. 128). Hence, the somewhat arbitrary nature of the separation for descriptive purposes, of these two regions, should be constantly borne in mind.

After 7 cm., the parietals are no longer confined to the deepest part of the fundic tubule, but invade all but the upper quarter. Some groups contain 10–12 cells. Especially characteristic from this time on is what we may call the "lateral parietal row." This is a row of parietals, one or two abreast, extending, often, from a point $\frac{3}{4}$ up the tubule, either to the very bottom, or only part way down (fig. 15). The occurrence as a row is, of course, determined only by reconstruction and by cross sections, and has been often verified thus. In cross section such a tubule will show one or two parietals, all the others being adelomorphs. These lateral rows occur even in the last stages before birth. However, the haphazard distribution of parietals ordinarily described is also found, isolated parietals, or groups of 2–3, or even 10–12, occurring from now on, not only in rows, but also in superficies. Fig. 13 represents serial sections through a gland-tubule of 10 cm., and illustrates the *partial* differentiation of the fundic elements into parietals, staining a brilliant red in fuchsin S.

The ductules in relation to the parietal cells are present at all subsequent stages, and hereafter, in this paper, their presence will be taken for granted. Often a ductule ramifies, sending branches between three or four parietals, or two branches between the same two parietals. These intercellular ductules are limited by cement lines, as shown beautifully by neutral gentian. Most of the parietals, at 8 cm. and later, display clear vacuoles, sometimes as clear areas surrounding the nucleus, but often lying in the peripheral cytoplasm. Harvey ('07) Hamburger and others describe these in the adult parietal (*vide* figs. 18, 20). In general, they become more constant, numerous and large in the later stages. Of course the obvious inference is that they represent droplets of secretion to be poured into the intercellular ductules. I have never





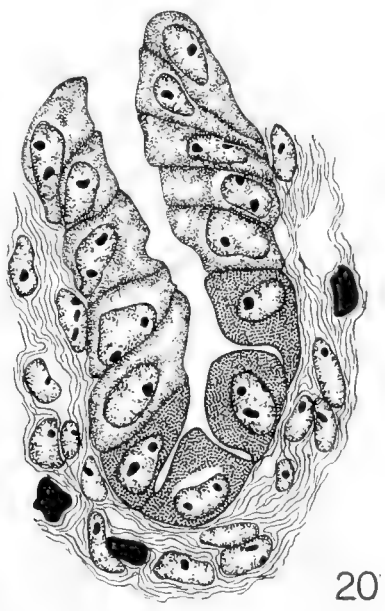


Fig. 18. Cardia. 19 cm. Three color. The cells represented in outline are surface mucous cells.

Fig. 19. Fundus. 19 cm. A complete tubule. Three color.

Fig. 20. Fundus. 19 cm. Bottom of a tubule, showing parietals and adelomorph cells. Copper chrome hematoxylin.

been able, by Golgi impregnation, although I have tried many times, to demonstrate, even in the latest embryonic stages, any continuity between these vacuoles and the ductules.

After 7 cm. mitoses are very frequent in the fundus tubules, occurring at all depths, but especially at the very bottom and at the juncture of the first (upper) and second quarter, *i. e.*, in the territory corresponding in the adult, to the lower part of the foveola. Most are in the adelomorphs, nevertheless parietals are often caught in mitosis. At 8–9 cm., every tubule of the fundus region has acquired some parietal cells. At 9 cm., and later, parietals are found with constricted nucleus. These I interpreted as examples of amitosis. Possibly they presage a cessation of cytoplasmic division. In later stages (20–29 cm.) multinucleate parietals appear. After 10–11 cm. the actual and relative increase of parietals in the cervical region, and relative decrease in the bottom of the tubule is marked. The latter is due to the proliferative activity of the undifferentiated gland cells, by which the tube is constantly lengthening downward. Mitoses also occur on the processes but not so frequently.

At 9–10 cm., no mucus is present in any fundic cell. At 12-13 cm., this is yet true for the large part of the fundus, but, near the pylorus, along the greater curve, very shallow, distal goblets of mucus appear in the cells of the surface and upper quarter of the tubule. At 15 cm. in this part of the fundus, the surface cells have acquired quite deep goblets, while in the upper third of the tubule are shallower goblets, and a few mucous chief cells. In the stages 15–20 cm. this mucous differentiation of surface and foveolar cells spreads rather gradually to the other parts of the fundus.[11]

Thus, even at 17 cm. we find a condition not far advanced, in this respect, over that of 13 cm.,—the surface and foveolar cells of some areas presenting but narrow rims of mucus, and the neck chief cells staining but faintly, or not at all, in muchematin.

[11] This occurs with striking slowness as compared with its rapidity in the pylorus. Moreover, in the latter, the gland cells at the very bottom were the first to manufacture mucin, the pyloric surface cells of some embryos showing no mucus as late as 12–13 cm, but the gland mucus chief cells staining heavily.

Fig. 15 represents the appearance of an average fundic gland of 12 to 16 cm., but it must be remembered that reconstruction shows a preponderance of parietals in the 2d and 3d quarters, over the number in the deepest fourth, also that in some parts of the fundus the surface and upper tubule cells may have acquired shallow goblets, which appear clear and transparent in the three color stain. It will be seen that the tubule has been lengthened by upgrowth of the gland processes. The latter are often clubbed or expanded at the tops and sometimes irregular. The upper $\frac{1}{4}$ or $\frac{1}{3}$ of the tubule devoid of parietals, and with cells already sometimes bordered with mucus, represents the future foveola. The rest of the tubule contains the cells from which will be derived all the epithelium of the adult cervix and fundus. Already (12–16 cm.) the parietals are preponderating in the middle third of the tubule, while the embryonic gland cells, in the deep $\frac{1}{3}$, are multiplying rapidly mitotically, thus deepening the tubule, and incidentally furnishing the material from which, as we shall see later, the zymogenic cells are to differentiate. For the present, these adelomorphs are, in every way, as far as the staining methods at my disposal show, the same as the primitive, embryonic gland cells. They have become however, somewhat more definitely cubic or low cylindric.

This description of tubules applies to the large part of the fundus, including those parts near the pylorus. In the fundic areas toward the cardia, we find that after 11–12 cm. not only are the foveola and surface mucous cells present, but occasionally cells in the deeper parts of the tubule tinge definitely with muchematin. At 14–15 cm. some of the tubules are lined to the bottom by mucous and parietal cells, the mucus cells in the depths being of the mucous chief type, those in the upper third shallow or deeper goblet cells. Interspersed with such tubules are some with three cell types—mucous, parietal and adelomorphs; and these tubules predominate more and more in progressing toward the right, until at last the typical tubules are reached. These left fundic tubules have as far as cytodifferentiation is concerned, reached the definitive condition. They constitute in embryos as in adult, a zone transitional, in all respects, between cardia and fundus.

In embyro and adult there is found, in passing through this zone from left to right, a progressive decrease in mucous chief cells, an increase of embryonic gland cells (or, in the adult, of serous chief cells) a gradual lengthening of the whole gland, and a gradual relative shortening of the foveola and lengthening of the fundus segment.

Thus at 17–19 cm., in the typical fundus area, the bottom of the tubule is lined by adelomorphs and parietals, the former preponderating. The former do not tinge with muchematin, up to the time of their final conversion into zymogen cells, for such will soon be their fate. In other words, those cells destined to become the serous chief cells, do not pass through a mucous stage, but pass directly from the embryonic form to the complete zymogenic cell. Up to 19 cm., neutral gentian, aside from tinting the cytoplasm of all the cells a rather faint, diffuse violet or blue, stains no granules, except the moderately course ones of the parietal cells. At 19 cm. granules of a new type appear in the distal zone of those cells of the fundic segment of the fundic glands, which have hitherto preserved the embryonic type. These granules stain, from the first, a dense blue. At the time of their appearance they are slightly larger than the parietal cell granules. They do not appear simultaneously in all the adelomorphs, but at first in scattered ones, spreading rapidly to the other cells of this type, so that, at 25 cm. no embryonic gland cells remain in the fundus, all having differentiated into serous chief or zymogenic cells. The granules increase rapidly in size, so that they are soon much larger than those of the parietals (fig. 22). In some cells, from the first, they are not limited to the distal zone, but occur throughout that part of the cytoplasm distal to the nucleus. The cytodifferentiation of the fundic glands seems now complete.

The remaining changes are merely such growth processes and shifts in the relative size and position of parts, as are necessary to bring about the definitive form and positions. As these undoubtedly display much generic, or even specific variation, they can have no general developmental significance, such as possessed by the details of cytodifferentiation, and will, therefore be described but briefly.

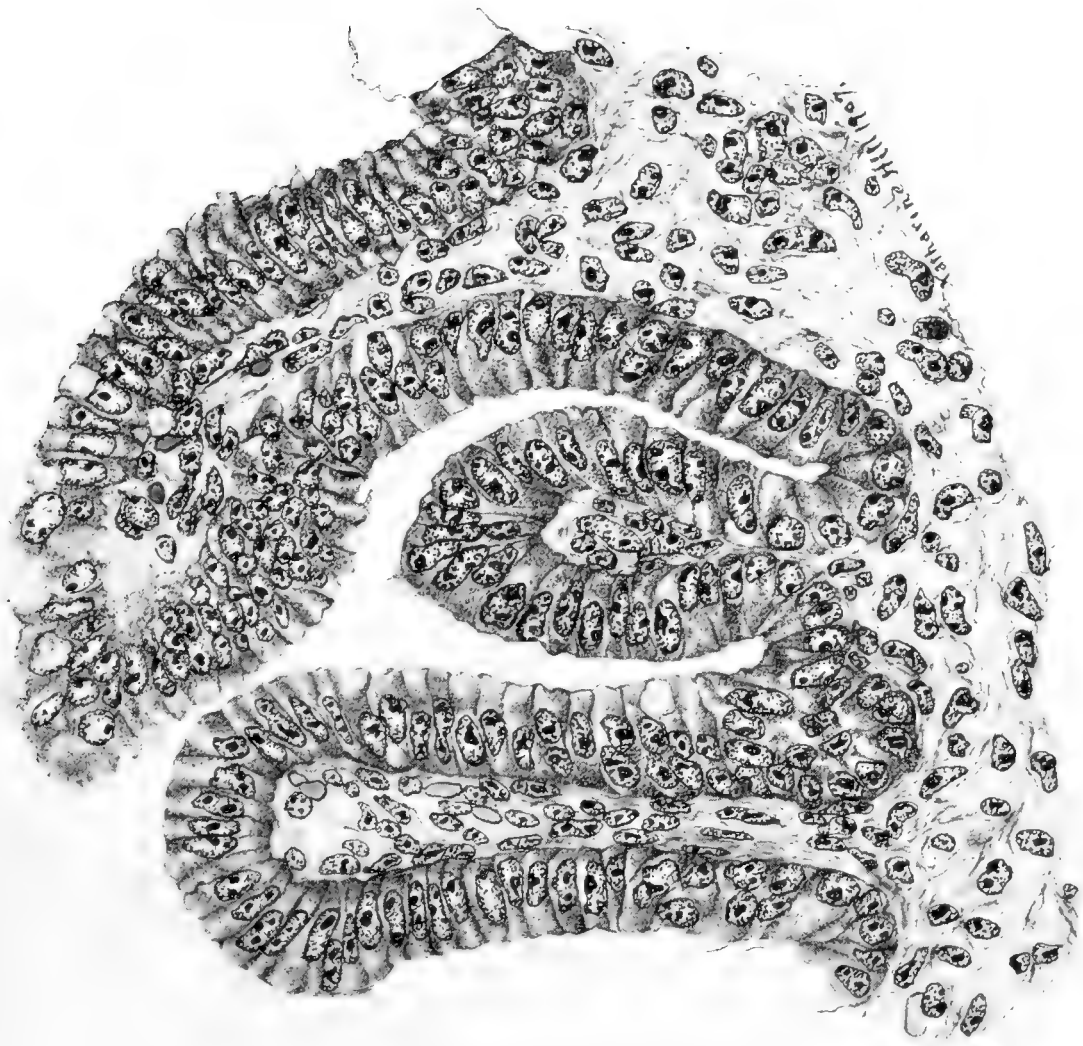


FIG. 21. Pylorus. 19 cm. Three color.

In cross sections of tubules at 19 cm. most of the parietals are shown to be in line with the other cells (fig. 23) but here and there a few are assuming the true parietal position. At all stages of this process, the ductules preserve their direct continuity with the gland lumen. The parietals here, as in the cardia, are, by the 24 cm. stage, much larger than the chief cells and of a rounded or ovoid form.

At 19–20 cm., by the progressive upgrowth of the gland process the foveola (part above the region of parietals) has lengthened very perceptibly, so that now it constitutes about one-half the total tubule length (fig. 19). The deep goblets of the foveola (a, fig. 19 are replaced below, in the cervical region, with mucous chief cells (b), with which parietals (c) are interspersed. The lower third is lined by serous chief cells, adelomorphs and a few parietals. The adelomorphs and serous chief cells are not distinguishable in the three color stain of fig. 19. Both are in frequent mitoses.

After 19 cm. by elongation of the tubule part (cervix and fundus segments) the proportions of tubule length and foveolar length are rapidly altered so that at 25 cm. the foveola : tubule : 1: 2. This proportion is preserved up to the time of birth.

4. *Cardia and caecum*

At 7–9 cm. the cardiac glands are mostly either in the intra-epithelial stage or in stages between those of figs. 3 and 6. Almost all, except the very youngest, have one or two parietals. The cardiac glands have now extended to the summit of the caecal swelling, being here of the intra-epithelial type. At 7 cm. all the cells are either adelomorphs or parietals. The later are at first, as in the fundus, confined to the lower part of the tubule (fig. 11) but at 10–11 cm. appear higher up, as in the fundic tubules of 7–8 cm. Lateral parietal rows soon become very common (13 cm.)

At 11–12 cm. and thereafter, differentiation of the surface cells into mucous cells occurs. Some acquire a mere distal rim of mucus, some a deep goblet. At 13–14 cm. the mucous change

extends into some of the tubules, sometimes to the very bottom. These deep mucous cells are then of the mucous chief type, the whole cytoplasm distal to the nucleus being infiltrated by the stainable substance. Thus, mucous differentiation occurs much later than in the pylorus, and somewhat earlier than in the fundus. However, the process lacks uniformity, for, as late as 14–15 cm. the cardia of some embryos exhibits mucous cells only on the surface, and a short distance into the tubule, with only an occasional one in the deeper parts of tubules.

The parietals, are, of course, unstained in muchematin, but are, as always, readily distinguishable (as, indeed, even in unstained specimens, fixed in Bensley's fluid) by high refractility, absolute opacity, and glistening shiny appearance. Moreover the ductules are very apparent. The adelomorphs are clear and transparent, though very finely granular.

At 17–18 cm. all the cardic cells are either mucous or parietal. The cells of the surface, for a little way into the tubule, possess shallow to deep goblets. The deep cells, aside from parietals are all of the mucuos chief type. Fig. 14 illustrates the typical surface goblet cell (a) and the shallow goblets (b), which go over by gradual transitions into the deeper mucous chief cells (c). In some tubules the goblet cells extend quite to the bottom but it should be noted that, in embryonic stages, the distinction between mucous chief and goblet cells is often not so sharp as in adult life, for, in the surface and upper tubule cells, the mucus often seems not to be homogeneous, but separated into minute goblets by a cytoplasmic mesh. Karyokineses are frequent in the mucous chief and goblet cells. Even the deep surface goblet cells are found in mitosis frequently.

From 14 cm. on, a marked form-divergence is added to the difference in size between cardiac and fundic tubules, the former now becoming shallower (wider relative to the depth), while the fundic tubules preserve their accustomed narrower, deeper form, so that, after 14 cm. it is easily possible to distinguish the two under low power (compare figs. 16, cardia, with 15 and 22, fundus). The cardiac epithelium has, from now on, a curious tendency to shrink away from the underlying mucosa, surface

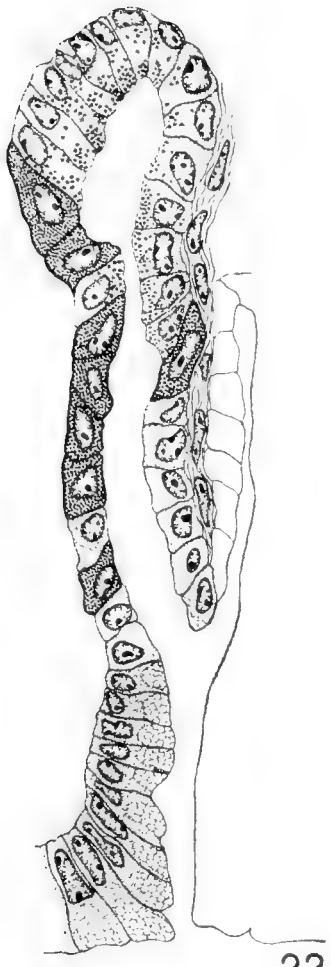
epithelium and tubules cohering so that the whole epithelial area loosens en masse. This tendency is observable from 14 to about 20 cm. in almost all stomachs, and is doubtless referable to the shallowness of the pits, taken in conjunction of course, with the fixation shrinkage.

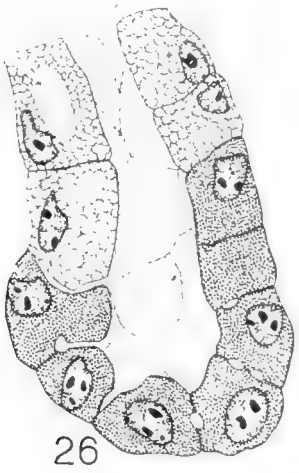
The upper $\frac{1}{3}$ or $\frac{1}{4}$ of the primary cardiac tubule, devoid of parietals and lined by goblet cells, represents the adult foveola, for the lineage may be traced, without any break of continuity, to the 29 cm. stage, long before which all the definitive parts of the tubule are readily distinguished. The lower $\frac{2}{3}$ or $\frac{3}{4}$, lined by groups of parietals, interspersed with a large number of mucous cells, gives rise later (20–22 cm.) to the tubules proper.

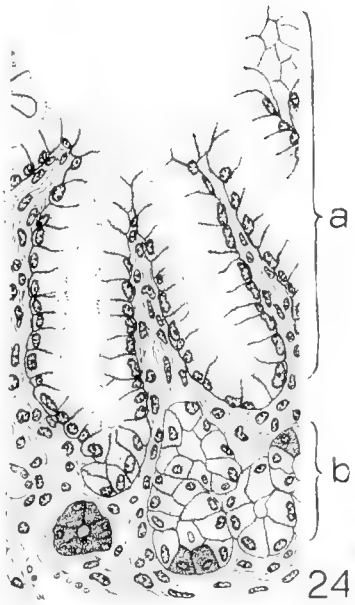
At 15 cm. certain of the groups of parietals begin to push outward slightly (fig. 16). These invaginations, at first shallow, may occur wherever groups of parietals occur,—namely, in the depths of the tubule, and in all but the upper quarter. At all stages they consist entirely of parietals. They appear with increasing frequency in the later stages, and become definite, secondary tubules (figs. 17 and 18) by 18–19 cm. Those from the sides of the primary tubule are directed outward and downward; those from the bottom, straight downward. These secondary tubules are narrower than the primary ones, and at 19 cm. vary from the merest incipient outpouchings to complete, though short, tubules. No similar process occurs in the fundus or pylorus at any stage.[12]

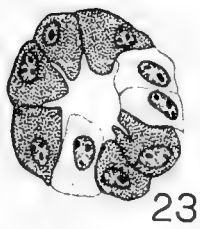
About 20 cm., the compounding of the lower part of the primary tubule occurs in the usual way, so that, at 22 cm., several tubules open into a foveola. Some of these tubules are purely of parietal cells; these were partially derived from the parietal evaginations. Some are purely of mucus cells. Many display both types, and in all proportions. These three types of cardiac tubules persist up to the time of birth (29 cm). I do not believe that the formation of these parietal tubules differs in essential mechanism from the or-

[12] This unexpected finding agrees absolutely with Toldt's observation in the cat, that some tubules arise as lateral buds, which grow out and downward. They arise at a parietal cell, and the latter by division goes over into the new tubule.









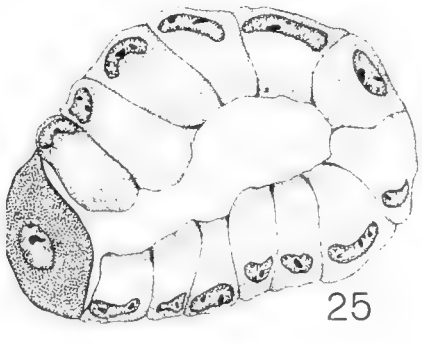


Fig. 22. Complete tubule of fundic region. 23 cm. Neutral gentian. Cyto-differentiation is practically complete. (29 cm is about the birth-length).

Fig. 23. Cross section of fundic tubule. 23 cm. Three color.

Fig. 24. Cardia. 25 cm. *a*. Foveolae. Mucous chief and goblet cells. *b*. Tubules. Mucous chief and parietal cells.

Figs. 25 and 26. Cross and longitudinal sections taken at the bottom of a cardiac tubule. 29 cm. (just before birth). This shows the parietals, which later, by the third week of post-uterine life, disappear or become converted into mucous cells.

dinary process of compounding, found in fundus, pylorus and also in the cardia. In each type, the epithelium seems to display the initial irregularity, but soon mesoblastic growth seems to become the active factor. I wish to emphasize the point that there are never, at this time, any transitions between the parietals and the mucous cells, each of which are, in the cardiac tubules, sharply distinguishable by the criteria mentioned above.

While the lower part of the tubule lined by mucous chief and parietals, is being compounded, a very rapid growth of the gland processes elongates greatly the wide, upper part lined by goblet *i.e.*, the foveola (19–25 cm.) Thus at 18 cm. (fig. 16) the foveola constitutes only the upper third or fourth. At 22.5 cm. the measurements are, foveola 48μ, tubule 64μ, total 112μ or foveolar length to tubule length as 3 to 4. At 25 cm. the foveolar length is to the tubular length as 5 to 3, and the total length is 140μ, so that the tubule portion has scarcely lengthened since 22 cm. Thus the definitive form and proportions are practically reached at 25 cm. (fig 24) and from now on, foveola and tubules grow with about equal rapidity.

As in all earlier stages, there is, at 25 to 29 cm., a gradual transition from undoubted cardiac to undoubted fundic tubules, the foveolae, in approaching the fundic region, becoming relatively and absolutely shorter, but the tubules so much longer that the whole gland length is greater.

At 29 cm. the cardic tubule measures 160μ in length, the undoubted fundic 210μ, and the pyloric 240μ.

In stages 26–29 cm. the parietals are just as numerous and just as well defined (figs. 25 and 26) as ever, staining with normal brilliancy, and possessing ductules and all the characteristic structures. But the cardiac glands of the adult pig contain no parietals, only mucous chief and thecal cells. Thus the parietals must either degenerate, or become transformed into mucous cells, during the intervening period. I have been unable to obtain stomachs of young pigs. I suspect, from the results of Cade and Harvey ('07) that the parietals may transform into mucus cells. I have to report on this in a subsequent paper.

One more point in the cytodifferentiation of the cardia should

be mentioned. The neutral gentian stain was used on every stage of cardiac development. This was done purely as a matter of routine, as I never anticipated finding anything in the cardiac tubules staining specifically with it, except the copper red granules of the parietals. But at 25 cm. and thereabouts granules resembling zymogenic granules appear in the deeper cells of undoubted cardiac tubules. Adjacent sections stained with muchematin show these same cells to be mucous chief cells of the ordinary type, so that the granules lie within the mucous parts of the cell. These granules are found intracellularly for one or two (cm.) stages, and after that disappear. I did not find them in the extreme left cardiac region.

At birth, the cytodifferentiation of the cardia is incomplete, or, rather, that involution required to bring about the adult cytologic status, with its single type of gland cell, has not yet begun.[13]

Caecum. At 10 cm. the glands are rather sparsely distributed. Many are as deep as the pyloric tubules, but contain few or many parietals. By 15 cm. the glands are almost as thickly distributed as in cardia or fundus, the parietals are abundant, and the tubules have the general characters of those of cardia and fundus, approaching more the deeper form of the fundic tubules. In later stages this region undergoes the same change as the general cardia.

5. *Pars oesophagea*

The greater part of this region gradually becomes stratified during the 6 to 10 cm. stages, the cells still remaining clear and transparent, except those of the deepest layer, which have acquired a finely granular cytoplasm of the ordinary type. The clear cells of the superficial strata have already begun to flatten out. At the periphery of the stratified area, the columnar cells constituting the deepest layer, are continuous with the simple columnar epithelium of the surrounding zone. We have seen that at 4–6 cm. a narrow, peripheral zone of this region has developed gland

[13] Negrini, 1886, noted that parietals are present in the foetal, but absent in the adult cardia; also Bensley, 1903.

tubules. These glands display the same form-development as the others. They are more precocious than the cardiac tubules, and deeper progressively, keeping pace with the pyloric tubules in development. At 11 cm. they still consist of clear, transparent cells. At this stage, then, we find the major and central portion of the pars oesophagea to be of stratified squamous epithelium; outside this is a zone of simple columnar epithelium which displays the glands at the very periphery. At 11 cm. the boundary between the clear cells of pars oesophagea and granular cells of adjacent cardia, fundus and pylorus is sharp to a cell. I have not been able to settle definitely the fate of this glandular zone in later stages. It seems, by differentiation of the cells, to merge with the adjacent cardia.

6. *Conclusions and theoretical deductions*

a. Homologies and phylogeny of the cardia

We have seen that fundic and cardiac glands display an absolutely parallel development in the earlier stages, the cardia, however, lagging behind, from the first. In each the parietals appear at a corresponding stage. It is impossible to distinguish an isolated 8 cm. fundic from a 12–13 cm. cardiac gland. After 13–15 cm. however, development in the cardia is retarded; while the fundic tubules elongate rapidly and become narrow, the cardiac tubules lengthen but slowly, and assume a shallow contour.

At the same time a significant parallelism appears in the cytodifferentiation. The foveolar region of each, with goblet cells, appear to be, at all stages, homologous. The lower part of the 16–17 cm. cardiac tubules corresponds, cytologically, to the neck region of the young fundus tubule, each being composed of mucous chief and parietal cells. The length of the whole cardiac tubule at this stage, is about that of foveola plus the neck of the fundus gland.

In the fundic gland of 20 cm. the body and neck segements become compound. In the cardiac gland of 25 cm. the body segment is evidently absent, barring the very temporary appearance

of a few zymogenic cells in the deepest part, but the neck segment, like the corresponding part of the fundic gland, has become split up into several tubules, which display the two cells characteristic of the neck of the fundic gland. While the aberrant development of zymogen granules is confirmatory, I lay most emphasis on the early parallelism in form-development, and especially on the presence, distribution and remarkable persistence of parietals in the cardiac tubules, as demonstrating their affinity with the fundic tubules.

By all these findings we are inevitably brought to one conclusion with reference to the genetic relation of cardia and fundus. *The cardia represents a part of the fundus which has undergone partial involution.* The regressive changes have manifested themselves first in the general chronologic retardation of ontogenetic development in the left part of the old cardio-fundic region; second, in the phylogenetic disappearance of the fundic segment of the tubule with its zymogenic cells,—the curious, delayed appearance of zymogenic granules at one stage of the developing cardia representing, probably, the last vestige of this segment; third, in the ontogenetic transformation of the cervix of the old fundus gland, with its mucous and parietal cells, into the tubule of the adult cardia with its mucous chief cell. This last process I have not seen, but it must needs occur, for we have the first and last terms of the series before us.

The zone of fundic tubules, lying between undoubted cardia and the ordinary fundic zone, represents an intermediate stage in the regressive process.

The facts of development, then, show that the cardiac glands are not primitive, but regressive structures. For the early stages do not resemble, in any way, the condition found in lower vertebrates, where, for example, even the fundus glands possess no definitely identified parietals. Moreover, no fact in the developmental history so much as suggests a possible derivation from the oesophagus.

Thus, our embryological findings seem to confirm strongly the conclusion as to the origin of the cardiac glands reached by Bensley ('02), working from the standpoint of adult comparative

anatomy and histology. It has been pointed out that general, mucous differentiation occurs slightly earlier in the cardia than in the fundus, although it is less uniform. The cardiac glands, in their whole development, display a very decided mucous character, as manifested in the discarding of the deep, zymogenic segment of the fundic tubule, in the involution of parietal cell, and in the slight acceleration of the mucous differentiation. This must be a response to a functional demand. Bensley has already suggested ('02 p. 147) that in these retrogressive glands, the cellular types disappear in the order of their specialization,—zymogenics first, parietals next. We find that not only is there no tendency on the part of the mucous cells to disappear, but that they even differentiate at a slightly earlier stage. Probably the altered food, or the other conditions which have brought about the involution of fundic to cardiac glands, have also demanded an increased mucus secretion.

It will be recalled that, while part of the caecal glands were as retarded as those of the cardia proper, others kept pace with the pyloric tubules, both types, however, developing parietals. Later, all the caecal glands, whether precocious or retarded, shared the fate of the cardiac tubules. This would seem to indicate that seclusion in the secondary pouch has tended to partially protect these galnds from the regressive changes which have attacked the old left fundic area.

Bensley's comparative work on the mammalian stomach (Op. cit. '02) led him to the same conclusion,

b. Cell specificity

At 2 cm. all the cells are of the type described as "embryonic gland cells." From 3 cm. on, some of these are constantly differentiating into parietals, others retaining their undifferentiated character for a shorter or longer period. Both types multiply mitotically.

From 6 cm. on, certain of the adelomorphs differentiate into mucous cells, both mucous chief and goblet. New parietals and new mucous cells arise, differentiating from adelomorphs. The

residual adelomorphs of the deepest part of the fundic tubules differentiate at 19–20 cm. into zymogenic or serous chief cells, and then multiply mitotically.

Thus the parietals are the first of the adult cell types to appear. Since the completion of this work, Dr. Bensley has pointed out that we have here a very remarkable and extreme instance of the throwing back of a coenogenetic character (parietals are not definitely identifiable in lower vertebrates) into the earliest ontogenetic stage,—a process called by Cope and Hyatt "acceleration."

Phylogenetically, zymogen cells (*vide* Amphibia), and especially mucous cells, are much more primitive, yet they here appear in ontogeny later than the parietals. Dr. Bensley has suggested that the early appearance of the intracellular ductules perhaps points to a functional explanation of this otherwise puzzling ontogenetic anticipation.

Taken per se, the facts of cytodifferentiation, as recounted above, seem to throw no definite light on the problem of cell specificity. But studied in conjunction with Harvey's findings ('07) they point, I believe, very definitely to certain conclusions.

The cytodifferentiation proceeds, as if the cells all started with the same potentialities or character complexes. These might be represented by *a*, *b*, and *c*. In some cells those metabolic character complexes represented by *a* become, as development proceeds, dominant, while *b* and *c* are dormant. In other cells, character complex *b* may dominate, and so on. But the dormant character may, under experimentally (Harvey) or pathologically (Cade, etc., cited by Harvey) altered conditions, become the dominant ones.

The results of the present work seem to indicate that this latter process does not occur in the normal course of histogenesis. The embryonic cells differentiate directly into the definitive types which then give rise, by division, to cell generations of the same specialized type. Thus, if the zymogenic characters have become dominant in a given cell, during the cytodifferentiation, then the off-spring of this cell are all zymogenic, but, nevertheless, the dormant parietal and mucus characters are transmitted to

each new generation and are available, if altered conditions make it desirable or imperative that they should become dominant. This view of the morphologic flexibility of "specialized" cells, under changed conditions will be recognized as O. Hertwig's doctrine of the nature of cell specialization, under a slightly different guise. But this conception is also expressable in terms of Weismann's Keim-plasma theory, provided sufficient emphasis be laid on the accessory Keim-plasms.

It seems probable that the mucigenic character of the large proportion of cardiac mucus cells,—namely, those derived from the mucus cells of the foveolar and cervical segments of the old left fundus glands, is palingenetic. On the other hand, it is probable that some of the deeply situated mucous cells are coenogenetically mucous, having passed, in race history, through the zymogenic or parietal stages.

The temporary occurrence of zymogen in the deeper mucous cells seems to indicate this, and to suggest that here the reserve zymogenic characters have not yielded, without a struggle, to the mucigenic, the old dominance of the zymogenic having become through the habit of ages, too strongly impressed on the cell metabolism to be rapidly effaced by the coenogenetic dominance of the mucous characters.

Bensley ('00) found, in the developing of gastric glands of Amblystoma, cells which contained for a brief period, both zymogenic and mucigenic granules. Thus it seems that sometimes, in the embryonic and possibly in the adult stages,—the cell metabolism may be controlled by two distinct sets of determinants,—through the manifestation of either of which we are ordinarily inclined to consider a cell as "specialized." Such instances seem to be uncommon, and it is probable that sooner or later,—one metabolic complex takes a subordinate place,—at least in the metazoa.

The fate of the cardiac parietals, after birth, promises to be of the greatest interest in this connection, as there are only two possibilities open to these cells —degeneration, or conversion into mucous cells.

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THE DEVELOPMENT OF THE MUCOUS MEMBRANE OF THE ŒSOPHAGUS, STOMACH AND SMALL INTESTINE IN THE HUMAN EMBRYO

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WITH TWENTY-FOUR FIGURES

SEVEN PLATES

The development of the mucous membrane of the digestive tract, although studied for many years by competent observers, still affords opportunity for further investigation. The present work was undertaken for the purpose of studying the development of the structures found in the digestive tract, and to obtain a comparable series of wax reconstructions illustrating the changes that take place in the form of the mucosa. Especial stress has been laid on the development of vacuoles and diverticula, villi, glands, and folds. It was originally intended to include the development of the mucous membrane of the vermiform process and the large intestine, but for lack of favorable material, this has been omitted in the present paper. This work was suggested by Dr. F. T. Lewis, and has been done in connection with his chapter on the digestive tract for Keibel's Text Book of Human Embryology. A more complete review of the literature than is here given, will be found in Dr. Lewis' chapter.

The material used was obtained from human embryos in the Harvard Collection. The earlier stages were already prepared in the embryological collection. The crown rump lengths and series numbers of these are as follows:

Length in mm.	Series No.	Length in mm.	Series No.
7.5	256	23	181
10	1000	24	24
16	1322	29	914
19	819	30	913
19	828	32	649
22	851	37	820
22.8	871	42	838
22.8	737	78 (incomplete series)	723,724

The crown-rump lengths and numbers of the older embryos used are as follows:

Length in mm.	Series No.	Length in mm.	Series No.
55	249	120	203
73	116	134	30
78	142	145	131
91	224	187	315
99	340	240	186
120	342	Newborn	341

From the older embryos, the different parts of the digestive tract were separately removed, imbedded in paraffin, and cut in serial sections of 8 microns in thickness. The sections were stained by different methods, among which were Heidenhain's iron hæmatoxylin, Hansen's iron hæmotoxylin, and Mallory's aniline blue connective tissue stain. Both eosin and orange G were used as counter stains. From the preparations thus made, certain stages of development were selected, and a number of wax reconstructions made. The models have in most cases been made to the magnification of 145 diameters, so as to be easily compared. However, in some of the gland models, this magnification was doubled, and in others trebled.

ŒSOPHAGUS

Early Stages. In an embryo of 7.5 mm., the œsophagus is a cylindrical tube of epithelium extending from the pharynx to the stomach. The lumen is round and relatively large at its upper end, but tapers gradually until at its middle it is quite small. Passing downward from this region, the œsophagus gradually increases in size and at its lower end leads into the stomach, there

being no sharp line of demarcation between the two. The indistinctness of the cell boundaries makes it very difficult to determine definitely the number of cell layers in the epithelium. Because of this, different observers have not agreed upon the number of cell layers present in the early stages. It can, however, be said with reasonable certainty that the epithelium at 7.5 mm. is stratified and in the upper and lower thirds of the œsophagus shows three or four rows of nuclei, in the middle third, two or three rows. The basal cells are the more columnar and are characterized by having their nuclei in that end of the cell which is toward the lumen. Surrounding this central tube of epithelium is mesenchyma, which although undifferentiated is slightly condensed.

In an embryo of 16 mm. the œsophagus has increased in length and breadth. The upper end is both larger and more irregular in outline. The epithelium contains three or four rows of nuclei and lies on a very distinct basement membrane. Here again, the basal layer of cells is the more columnar and its rounded nuclei lie in the upper ends of the cells, that is, away from the basement membrane. In its lower portion, the œsophagus is smaller and oval in cross section. Throughout its entire extent the œsophageal epithelium is surrounded by mesenchyma which is limited externally by a narrow ring of deeply staining myoblasts, elongated in form, and so placed as to form the circular layer of the muscularis.

Vacuoles. The next stage examined was an embryo of 19 mm. (series 819). In this only about the lower half of the œsophagus was available for study. The epithelial tube is now irregular in outline, and presents within its walls numerous small cavities or vacuoles. Some of these are minute, others have a diameter 0.051 mm., which is greater than the diameter of the lumen itself, making it difficult to determine from cross sections which is lumen and which vacuole. In some places the vacuoles open directly into the lumen, in other places they are separated from it by a partition of epithelium. Fig. 1a is of a model of the œsophageal epithelium at this stage and shows its vacuolated structure. The portion of the œsophagus modelled was selected at a place just below the bifurcation of the trachea. In a second embryo

of 19 mm. (series 829) similar vacuoles are found, but are less numerous than in the above embryo of the same length. They are also more numerous in the lower portion of the œsophagus than in the upper.

At 22.8 mm. (series 871) the diameter of the œsophagus is nearly double that at 19 mm. The vacuoles are more numerous and, as shown in fig. 1b, communicate with one another and with the main lumen to a greater extent. In the upper part of the figure the œsophagus appears to have a double lumen. This appearance is due to the fusion of a number of vacuoles. The vacuoles are about the same size as those found in the preceding embryo but the lumen has increased in diameter. Although vacuoles also occur in the dorsal and ventral walls of the œsophagus, they are less numerous there than in the lateral walls. The few vacuoles found in the dorsal and ventral walls do not show in the figures; they are, however, of the same general character as those described.

In other embryos of 22 mm., 22.8 mm. (series 737) and 23 mm. the vacuoles are found to be less numerous in the upper half of the œsophagus than in the lower half. At 29 mm. and 30 mm. they have become very few; at 32 mm. only slight traces of them are found; at 37 mm. they have entirely disappeared.

Vacuoles of the nature described were noted by Schultz in 1897. Kreuter ('05) believed that they cause a temporary occlusion of the œsophagus, but Forssner ('07) showed by means of models that the main lumen is not obliterated. Schridde ('08) failed to find an occlusion at any stage and denied the presence of vacuoles but described epithelial bridges which arise by epithelial proliferation in circumscribed places. From a study of these structures by means of wax reconstructions, the writer is able to confirm the results of Forssner inasmuch as the lumen is not entirely occluded and vacuoles are present.

The exact cause and significance of the vacuoles are difficult to determine. Similar vacuoles are found in the œsophagus of pig, rat and rabbit embryos. They have been reported by Forssner in the hedgehog, where in a certain stage they lead to a complete occlusion. Vacuoles similar to those of the œsophagus are found in the walls of the stomach and the duodenum of human embryos.

Kreuter states that there is no reason to believe that the vacuoles are areas of degeneration for he was nowhere able to demonstrate evidences of a degeneration of cells followed by resorption. There can be seen, nevertheless, a few nuclei lying within certain vacuoles which stain less intensely and are more indistinct than the nuclei of the epithelium. It does not appear, however, that these occasional degenerating nuclei are responsible for the production of the vacuoles. It seems obvious from the arrangement and crowded condition of the surrounding nuclei that the vacuoles increase in size. As they become larger the epithelium between the vacuoles and the lumen, and between adjacent vacuoles becomes reduced to a thin partition. This partition eventually breaks through and makes the cavity of the vacuole and the lumen continuous.

Measurements of the thickness of the epithelial wall of the œsophagus are interesting in connection with the vacuoles. At a time when vacuoles begin to form, the epithelial wall of the œsophagus is composed of apparently three or four layers of low columnar cells. While vacuoles are present the number of cell layers varies from one to four. By a breaking through of the vacuoles into the lumen and a disappearance (or migration) of the more superficial cells, the epithelium is reduced in thickness. At 19 mm. and 22.8 mm. the epithelium averages .051 mm. while at 30 mm. only .030 mm. in thickness. This loss in thickness is soon compensated by a growth in the height of the individual cells, for at 42 mm. there are only two layers of cells, the average thickness of the epithelium being .043 mm. Two results of vacuolation are, therefore, a destruction of the more superficial layers of cells, and an increase in the size of the lumen of the œsophagus. This increase in the size of the lumen is proportionately great in the early stages but less in the older ones.

Folds. At an early stage the epithelium of the œsophagus becomes thrown into large longitudinal folds which are usually four in number. Later smaller folds develop at the bases of, or in between, the larger ones. These folds give to the œsophagus in cross section the appearance of a Maltese cross as was first noted by Koelliker ('61). Certain of the folds make their appearance

before others and show different stages of development in different regions of the same œsophagus. The exact time and order of the appearance of these structures was studied in a number of embryos with the following results.

As stated before, the epithelial tube of the œsophagus at 7.5 mm. is cylindrical in shape, very small at its mid-region, but gradually becoming larger when followed either up or down. In embryos of 10 mm. and 16 mm. the upper portion of the œsophagus is flattened ventro-dorsally, the middle region is circular but now more expanded, and the lower portion is flattened laterally. In the upper part of the œsophagus there is a slight infold of the ventral wall of the epithelial tube.

In the upper part of the œsophagus at 22.8 mm. (series 871) there is a ventral infolding of the epithelium. This is the direct downward continuation of that fold of the pharynx which gives to the latter, when seen in cross section, its characteristic crescentic shape. Following downward this fold is soon lost and the œsophagus becomes rounded. Another fold appears in the mid-region of œsophagus, this being an infolding from the dorsal side. Still further caudally, as the stomach is neared, a third infolding is found, this being left lateral in position. This lateral fold in an older embryo is seen to be directly continuous with the dorsal fold above. The folds found in this embryo are, therefore, really two in number, a ventral one which appears in the upper third of the œsophagus, and a dorsal one which is seen in the middle third and again as a lateral fold in the lower third. Since the study of the order of the appearance of these folds was made by wax reconstructions in a series of embryos, it was necessary to secure for modelling approximately the same level of the œsophagus in each specimen. The region selected was that immediately below the bifurcation of the trachea.

The œsophagus at 37 mm. in the region of the bifurcation of the trachea (fig. 2) is somewhat crescentic in cross section. Dorsally, facing the dorsal aorta, there is a deep fold which continues upward and downward and passes directly onto the stomach. In the lower third, however, it deepens and changes its position by moving through an arc of about 90° to the right. At 42 mm.

in the same region as the above (fig. 3) there are two distinct folds, a dorsal and a ventral. These folds, which I have designated as the first pair of primary folds, are continued downwards onto the stomach, but before reaching it are twisted to the right and occupy positions on the left and right sides of the œsophagus respectively. In the figure a second pair of primary folds can be seen, which, although indistinct at this level, are well marked below. In the region modelled and below it, the fold on the left side is the more marked, but above the right fold alone is seen, and the ventral and dorsal folds approach one another on the left side, making a three sided figure. At the extreme upper end of the œsophagus these folds become irregular and are broken up by the formation of other smaller folds. All the folds shown in fig. 2 are twisted as the stomach is neared, each occupying a position of about 90° to the right of its original position.

At 55 mm., both pairs of primary folds are well developed (fig. 4) and give to the lumen of the œsophagus in cross section the appearance of a Greek cross. These folds are also seen in embryos of 30 mm. and 42 mm. in the lower parts of the œsophagus. As seen in fig. 5, at 134 mm., these primary folds are augmented by secondary folds which develop at the bases of the former. Three such folds are seen in fig. 4, one of which is quite large. The same folds are found in embryos of 187 mm., 240 mm., and at birth.

By way of summary concerning the development of folds of the mucous membrane of the œsophagus, it may be said that the dorsal one is the first to develop completely. This is followed closely by the ventral fold. The second pair of primary folds develop from below upwards and have reached to the bifurcation of the trachea in an embryo of 42 mm. The secondary folds are less constant structures. They begin as thickenings of the epithelium between the bases of adjacent primary folds. In the upper part of the œsophagus, especially in the older stages, folds are very irregular. The reader is referred to Table 1, page 532 for measurements of folds of the œsophagus in different stages of development.

The fact that the epithelial folds of the œsophagus rotate

through an angle of about 90 degrees and always in the direction of the hands of a clock, seems to have some significance. Just what the cause of this rotation may be is debatable. Kreuter discards the idea that the lower part of the œsophagus may share in the rotation of the stomach. However, this may be its cause. This view is supported by the arrangement of the branches of the vagus nerves. These branches are also twisted at the same level as the œsophageal folds. It would, therefore, not seem improbable that the lower part of the œsophagus had been twisted along with the nerves by the stomach. Since the twisting of the stomach takes place before the appearance of the folds, it would necessarily have to be assumed that the lines along which the first œsophageal folds develop are marked out before the actual appearance of the folds themselves.

Ciliated cells. The presence of ciliated cells in the œsophagus was noted in 1876 by Neumann, who observed them in embryos of from 18 to 32 weeks. He described the epithelium as stratified, the superficial layer being made up of patches of both ciliated columnar and squamous cells. Schaffer ('04), confirmed by Jahrmaerker ('06) and Schridde ('07), states that the ciliated cells may extend through the thickness of the epithelium down to the basement membrane. Jahrmaerker has recorded the presence of ciliated cells in the œsophagus of an embryo at 44 mm. In an embryo of 42 mm. no ciliated cells could be found, but the preservation of this embryo was poor and the staining unfavorable for the recognition of cilia. In a specimen of 55 mm. ciliated cells are abundant. Here the epithelium of the œsophagus is composed of from two to four layers of cells with distinct cell boundaries. The basal cells are columnar and have their oval nuclei away from the basement membrane. In those places where there are not more than two layers of cells, the superficial cells are columnar, very granular, and ciliated. Where there are three or four layers of cells, only the lower layer is columnar, the upper layers being composed of polygonal or flat cells.

In order to study the distribution and number of the ciliated cells, wax reconstructions were made, the areas of ciliated cells being carefully painted on each wax section before piling. Fig.

6, from an embryo of 55 mm., shows one half of a model viewed from its inner surface. The stippled areas represent those covered with cilia. As can be seen, the larger areas of ciliated cells are on the folds and these areas include but few islands of polygonal cells. Between the folds there is a preponderance of squamous cells, which are interrupted by a few small ciliated patches. In the upper part of the œsophagus the ciliated areas are slightly larger than in the region modelled. A similar reconstruction was made of the epithelium of the œsophagus at 99 mm. (fig. 7). It shows that the amount of ciliated surface has actually and relatively increased and there are now more islands of polygonal cells on the folds.

In embryos of 120 mm., 134 mm., and 187 mm., ciliated cells in the œsophagus are observed to be distributed in about the same proportions as at 99 mm. At eight months the upper part of the œsophagus shows only a few small patches of ciliated cells. In a specimen at birth the areas are abundant but proportionately smaller than in the earlier stages, and the islands are now more widely separated. The islands consist of distorted columnar ciliated cells, the distortion probably being due to the pressure of the rapidly increasing polygonal cells, which are seen crowded closely against the islands and in places partly undermining them. In a child of two weeks (7 months premature birth) no ciliated cells can be found in any part of the œsophagus. Jahrmaerker and Schridde were unable to find ciliated cells at birth.

Glands. In regard to the glands of the œsophagus, there are two distinct types to be considered; the true or deep œsophageal glands, and the cardiac or superficial œsophageal glands. The former are found in the adult all along the œsophagus, with the exception of the lower 2 to 4 mm. of its length; the latter in the lower 2 to 4 mm. and also (70 per cent of cases examined by Schaffer) in that portion of the upper part of the œsophagus which lies between the level of the cricoid cartilage and the 11th tracheal ring.

In an embryo of 78 mm. (incomplete series 723 and 724) small patches or islands of glandular epithelium are found in both the upper and lower ends of the œsophagus. Schaffer ('04) described

similar groups of cells in an embryo of four months, at the level of the third or fourth tracheal cartilage, and Schridde ('07) found a group in an embryo of 105–110 mm. (16 to 17 weeks) at the level of the cricoid cartilage. Areas of glandular cells are abundant in the lower part of the œsophagus at 120 mm. Some groups are arranged as islands, others are evaginated so as to form small pockets. From the bottoms of these pockets budding glands are seen. In the lower part of the œsophagus at 240 mm. the glandular cells are more conspicuous. On the sides of the large folds, but chiefly at their bases, patches of secreting cells are seen forming small isolated islands, pockets, and grooves. The glandular epithelium is high columnar and is everywhere surrounded by stratified squamous and ciliated cells. The pockets form the ampullæ of the cardiac glands, from which numerous small buds have grown out. A model of such a gland, found at the bottom of a fold at the lower end of the œsophagus, is shown in fig. 8. The surface which is ruled in the figure indicates the simple glandular epithelium; the unruled surface, the stratified squamous epithelium.

The presence of the deep œsophageal glands was first noted in an embryo of 240 mm. Their beginnings are seen as small outgrowths of the stratified epithelium. These buds are found at the bases of the primary folds and upon the outer surface of the secondary folds. They are lined by a stratified squamous epithelium similar to that lining the œsophagus. In a specimen from the upper part of the œsophagus at 8 months, a gland was found which extended through the muscularis mucosae into the submucosa. Its walls were composed of one and two layers of cuboidal cells, but nowhere throughout its entire extent could secreting cells be seen.

At birth the œsophageal glands (fig. 9) have grown out through the muscularis mucosæ and lie in the inner part of the submucosa. The mouths of the glands are lined with a stratified squamous epithelium of three or four layers of cells which is directly continuous with the œsophageal epithelium. Gradually the stratified squamous epithelium passes into the double layer (in some places single), of low cuboidal cells which lines the excretory

duct. The excretory duct of the gland passes through the thick muscularis muscosæ at nearly right angles to the surface epithelium. On reaching the outer border of the muscularis mucosae, it bends to either side. Instead of pointing toward the stomach, as has been described in the adult œsophagus (Goetsch), glands may extend either upward or downward. The distal terminations of most of the glands are expanded and lined by a simple glandular epithelium of low columnar cells. In the gland A (fig. 9) the end piece shows a number of bud-like processes which are the beginnings of branches. The end piece of gland B is a simple curved expanded tube, the expanded portion being for the most part glandular. In the gland C there is no end piece at all, the whole gland being lined by two layers of non-secreting low cuboidal cells. In a study of the œsophageal glands of the pig, Flint has described a similar early stage, in which the glands contain no secreting cells. However, glands of this type are rather rare in the human embryo at birth, there being a preponderance of those with secreting cells and beginning branches.

In summarizing the development of glands of the œsophagus, it may be said that the first evidence of the cardiac glands is the appearance of small patches of glandular cells in the surface epithelium. These patches occur at both the upper and lower ends of the œsophagus and are seen as early as the third month. Later, by evagination, small pockets are formed from these patches. This is followed by a subsequent budding from the outer surface. Thus are formed a number of branches which open into a single pocket or ampulla. Cardiac glands never extend through the muscularis mucosae. The deep œsophageal glands, first seen in an embryo of 240 mm., are outgrowths of the stratified squamous epithelium, which after piercing the muscularis mucosae, bend to either side, and lie in the submucosa close to the muscularis mucosae. The secreting cells do not appear until after the gland is well formed, and are found only near its distal termination. Here at birth a number of budding processes, lined with a secretory epithelium, are seen arising from the end pieces. Thus the cardiac glands and the œsophageal glands differ in that, in case of the cardiac glands the secretory epithelium appears before the

gland formation begins, while in the œsophageal glands it does not appear until after the excretory ducts are formed.

TABLE 1

Oesophageal Epithelium (Mid Region)

Embryo	Thickness[1] of surface epithelium		Diameter of epithelial tube	Primary folds		Secondary folds	
Length In mm.	In Cell Layers	In mm.[2]	In mm.	Number	Height[2] in mm.	Number	Height[2] in mm.
7.5	3–4 (?)	.020	.043	0			
16.	3–4 (?)	.043	.148	0			
19.	1–4*	.051*	.163	0			
22.8	1–4*	.051*	.223	1	.010		
30	2–3	.030	.306	2	.056		
42	2–3	.043	.398	3–(4)	.089		
55	2–4	.051	.51	4	.094	1	.012
99	3–4	.051	1.01	4	.31	4	.081
120	4–5	.043	.90	4	.29	4	.054
187	4–5	.051	1.63	4	.47	3	.091
240			1.81	4	.58	4	.127
Birth	5–6	.056	2.26	4	.68	3–4	.163

[1] On tops of folds when present.
[2] Average of a number of measurements.
* Vacuolated stages.

STOMACH

For this portion of the present paper only the fundus region of the stomach has been studied, the development of the gastric pits (foveolae gastricae) and the gastric or peptic glands (glandulae gastricae propriae) being given chief attention. Koelliker ('61 and '79) regarded the gastric pits and glands as hollow epithelial processes which grow downward into the mesenchyma. Laskowsky ('68) confirmed by Schenk ('74) and Brand ('77), described glands as epithelial in origin, but believed that an upward growth of the underlying mesenchyma was the active factor in their production. Sewall ('79), working on sheep embryos, concluded that the first chief and parietal cells were epithelial in origin, but were later replaced by others of mesenchymal origin.

Toldt ('81) in a noteworthy study of the histogenesis of the gas-

tric mucosa in cat, pig, and human embryos, concluded that the glands are formed exclusively by downgrowths from the epithelium. Minot ('02) likewise describes an epithelial origin for the glands. Later, Strecker ('08) returned to the old idea of a mesencymal origin for the gland cells. The results of the present work are largely in accord with those of Toldt. However the present study deals mainly with the form of the mucous membrane and is based upon a series of wax reconstructions, while Toldt was concerned chiefly with detailed histogenesis.

Early Stages. In an embryo of 7.5 mm. the stomach is a well marked, spindle-shaped swelling, about .132 mm. in transverse diameter. It is placed with its long axis parallel to the long axis of the body of the embryo. Its epithelium is about .046 mm. in thickness and at the extremities of the stomach is composed of two or three layers of low columnar cells, at the mid region of higher columnar cells. As in the œsophagus, the basal layer of cells is the tallest, and the nuclei are placed at the upper ends of the cells. In an embryo of 8 mm., described by Jahrmærker, the nuclei of this strata were found in the basal ends of the cells, and this he pointed out as a distinction between œsophagus and stomach at this stage. The epithelium of the stomach is surrounded by loose mesenchyma which is limited, except at its mesenteric attachments, by the mesothelial lining of the coelom.

In an embryo of 10 mm. the stomach is more pyriform in shape and is now undergoing rotation to the right on its long axis. The lumen has greatly increased in diameter but the epithelial wall is of about the same thickness, measuring .048 mm. The number of strata of cells varies from two to three. Both the basal and the superficial layers of cells are distinctly columnar the latter showing distinct top plates. The nuclei of the basal layer are again in the upper ends of the cells, those of the superficial layer at the lower ends. The surrounding mesenchyma is slightly condensed, and a basement membrane is distinct in some places.

Gastric Pits. At 16 mm. the stomach is lined by an epithelium of high columnar cells of two or three layers, the entire thickness of the wall measuring .054 mm. The nuclei of the basal layer of

cells are again in their upper ends. The epithelium on the side toward the bursa omentalis shows several irregularities. These are vacuoles and pit-like depressions. The vacuoles are similar to those found in the œsophagus, but are smaller and far less numerous. The pit-like depressions are surrounded on their sides by the columnar cells of the epithelium. The nuclei of the cells which line the pits are rounded and are quite regularly placed around the lumen, so that the whole structure has somewhat the appearance of a taste bud. The pits measure about .009 mm. in width and about .028 mm. in depth. There are no corresponding outbulgings of the basement membrane made by these pits on the mesenchymal surface of the epithelium.

In an embryo of 19 mm. the epithelium of the stomach is of the same type as in the preceding embryo, having two or three layers of cells and a thickness of .048 mm. It contains a few small vacuoles. The pits are more numerous than before and some are elongated so that now they form short grooves. These are most numerous in the lower end of the stomach. Again there is nothing on the mesenchymal side of the epithelium to correspond to these depressions. In this embryo the beginning of the tunica muscularis is first seen.

The epithelium of the stomach at 22.8 mm. measures on the average .055 mm. in thickness. A few vacuoles, similar to those found in the œsophagus of this embryo, were observed. They were, however, much smaller than those in the œsophagus. The pits are more numerous and distinct than in the preceding embryo. Again some are rounded and some groove-like in form, but still they produce no swellings on the under side of the epithelium. At 42 mm. the pits are abundant. The epithelium measures .055 mm. in thickness and shows for the first time slight bulgings into the mesenchyma.

In an embryo of 55 mm. the epithelium of the stomach shows well defined pits and furrows on its inner surface. A surface view of a portion of the epithelium of the fundus of the stomach at this stage is shown in fig. 10. The cylindrical pits are irregularly scattered about, and the grooves are placed parallel to the long axis of the stomach. The epithelium, which was previously

stratified, is now becoming one-layered. This change is brought about by a readjustment of the cells, caused—first, by the development of the pits; second, by the development of somewhat similar pits or furrows on the outer surface of the epithelium. These latter structures are found alternating with the pits of the inner surface and are in different stages of development. Some appear as mere slits in the epithelium, others are already broader than many of the gastric pits themselves. Whether these pits are formed by an ingrowth of the mesenchyma, or whether they are formed independently of mesenchyma by a readjustment of the epithelial cells, is difficult to determine. In the specimens studied, the mesenchyma had shrunken away from the epithelium, but from their extreme depth and narrowness it is hardly probable that the mesenchyma could have extended into the narrowest of these furrows. However formed, these furrows cause the epithelium to be transformed into a simple epithelium. The cells of the surface epithelium and those about the pits give off slender basal processes. These cells have been described by Baginsky ('82) at 7 months, and by Fischl ('91) at birth. The cells have distinct cell boundaries, rounded nuclei, are granular, and show distinct top plates.

At 91 mm. the grooves on the mesenchymal side of the epithelium are well developed. In a model of the epithelium at this stage (not figured) these mesenchymal grooves have about the same appearance as the gastric pits, so that one can hardly determine which is the internal and which the external surface. The epithelium is composed of a single layer of cells. The cells are now higher, with more elongated nuclei, and have long basal processes. Some of the cells are clearer than others and seem to be filled with mucous secretion.

At 120 mm. the gastric pits have increased in number and in size, and are slightly more separated from one another than before. The growth of the epithelium has taken place both by an enlargement of the pits already formed and by the addition of other pits. A wax reconstruction of the epithelium of the fundus of the stomach at this stage (fig. 11) compared with fig. 10 (55 mm.) will give a clearer idea of the growth of the pits. The arrangement

of the parallel grooves is beginning to be lost, and the whole surface of the mucous membrane appears to be cut up by the network of anastomosing pits. The areas marked off by this network are slight elevations (much higher in the pyloric region), and have been considered by Brand as villi. Those of the fundus, however, are much broader and more irregular at their bases than the intestinal villi. Moreover, they have an entirely different developmental history. It is doubtful, therefore, whether these structures of the fundus can be considered as villi. The tunica propria, composed of developing reticular tissue, extends up between the pits to the surface epithelium. The surface epithelium is composed of a single layer of columnar cells, which are filled with mucous and contain at their lower ends oval or irregular elongated nuclei. The basal processes are not so elongated as in the preceding stage. At the bottom of the pits the cells are cuboidal.

Gastric Glands. A view of the under surface of the epithelium at 120 mm. (fig. 12), shows the beginnings of the gastric glands. They appear as knob-like outgrowths from the bottoms of the gastric pits. As many as three glands are sometimes seen arising from a pit at the same level. The small buds are for the most part as yet simple glands, although several were seen which had begun to branch. They are of different lengths, the longest being about .048 mm., and their diameters at the necks measure about .036 mm. The glands show small but well defined lumina and are lined by a simple cuboidal epithelium. The parietal (delomorphous) and the chief (adelomorphous) are easily distinguishable. The parietal cells stain more intensely with eosin than do the chief cells, and their protoplasm is slightly more granular.

Cross sections through the middle of the stomach at 187 mm. show the mucous membrane to be thrown into a number of large folds. The gastric pits are of about the same character as described for the 120 mm. embryo but they have increased in size. Some of the largest now measure about .089 mm. in depth. The surface epithelium is higher than in the preceding embryo and is composed of cylindrical cells which are mucous in character. The cells contain oval nuclei which lie in the lower halves of the cells

but not against their bases. At the bottoms of the pits the glands are seen. These are now longer and more branched, the longest measuring about .12 mm. The diameters at the neck vary somewhat, but most are about .038 mm. The diameters of the branches are smaller than the main stem of the gland. The glands are lined by a simple epithelium of irregular or polyhedral cells. Both the chief and the parietal cells are abundant. The bases of the glands lie close to the muscularis mucosae, which at this stage is well developed and consists of several distinct strands of smooth muscle fibres.

The changes that take place in the stomach epithelium from the 187 mm. stage are merely those in the increase in numbers and growth of the structures already formed. At 240 mm. the gastric pits measure from .102 to .158. mm., and the glands about .85 mm. to .136 mm. in length. From this it can be seen that the growth of the glands has been more rapid than that of the pits, some of the glands now being longer than some pits. In a model of the epithelium of the fundus of the stomach at 240 mm. (not figured), gastric pits are more irregular in form but are broader across their tops than those of the 120 mm. stage. They are, moreover, further apart than before. Fig. 13 shows gastric glands modelled under higher magnification. Two types of glands are seen, the smaller simple ones and the larger branched. In the compound glands the branching is not confined to any particular level, but branches are given off at the neck or any level below it. The cells lining the pits are like those found in the preceding stages, the chief and parietal cells being more numerous than before.

At birth the structure of the mucosa of the stomach is very similar to that found in the adult. The gastric pits are only slightly deeper than at 240 mm. The surface epithelium is composed of high cylindrical mucous cells with oval nuclei and basal processes. At the bottoms of the pits the cells are lower but are still distinct. As seen in fig. 14 the glands are longer and more branched than before. They have small lumina which are lined with chief and parietal cells.

The growth of the mucous membrane of the stomach is perhaps best shown in table 2. From the earliest stage up to 42 mm. there is but a slight increase in the thickness of the epithelial wall. At 16 mm. the pits begin to form, and rapidly increase in number and in size. At 55 mm., due to the presence of pits on the inner surface and alternating grooves on the outer (mesenchymal) surface, as described above, the epithelium becomes reduced to a single layer of low cells. When measured as in the preceding stages, the epithelium averages .057 mm. in thickness. When determined as in the following stages, *i.e.*, the epithelium which surrounds the individual pit, it measures .012 mm. Thus by the readjustment of the cells which is completed at this stage, the epithelium is reduced in thickness from .057 mm. to .012 mm. At 91 mm. the surface epithelium is much thicker because of the extremely long basal processes of its cells. At 120 mm. the basal processes are shorter, but from this stage up to birth there is a gradual increase in the thickness of the epithelium. The gastric pits rapidly increase in depth but there is a more gradual and variable increase in their width and the distance between adjacent pits. The glands also increase rapidly in length, but slowly in diameter.

Folds. Folds of the mucous membrane of the stomach are more variable in number than in the œsophagus. In an embryo of 10 mm. the epithelial wall of the stomach is perfectly smooth throughout. At 16 mm. there are two or three slight folds on the side of the greater curvature, and indications of about the same number of the side of the lesser curvature. At 19 mm. there are 2–3 large longitudinal folds on the side of the greater curvature, while the opposite wall is rounded, with no indications of folds. At 22.8 mm. there are 7–10 folds, including both walls; at 42 mm. 10–12; at 55 mm. about the same number but they are not as high. At 120 mm. and 187 mm. large folds are found, while at 240 mm. and at birth the folds are very much higher. In the last four cases, the number of the folds was not obtained. From these and other observations it would seem that the folds are rather inconstant structures, their number and size depending on the state of contraction of the muscular walls. Brand ('77) found

12–15 longitudinal folds of the stomach mucosa in an embryo of two months. Koelliker ('69) found in an embryo of four months, 3–4 folds; in another embryo of four months, 11–12; at three months, 5–6. He therefore regarded their number as variable. Toldt ('81), in a study of cat embryos, considered the folds of the stomach as unimportant, being formed by the contraction of the musculature.

TABLE 2

STOMACH EPITHELIUM (FUNDUS)

EMBRYO	THICKNESS OF SURFACE EPITHELIUM		GASTRIC PITS			GLANDS		FOLDS	
Length in mm.	In Cell Layers	In mm.[1]	Depth[1] in mm.	Width (at tops) in mm.	Distance apart in mm.	Length[1] in mm.	Diameter[1] (at neck) in mm.	Number	Height[1] in mm.
7.5	2–3	.046							
10	2–3	.048							
16	2–3	.054	.028	.009	‡			5–6	.042
19	2–3	.048	.031	.009	‡			2–3	.067
22.8	2–3	.055	.048	.012	‡			7–10	.18
42	1–3	.055	.041	.012–.017	.036–.048			10–12	.25
55	1	.057 or .012	.048	.007–.012	.024–.036			10–12	.11
91	1	.048	.072	.012–.030	.048–.084				
120	1	.029	.084	.019–.036	.054–.108	.048	.036		.36
187	1	.036	.089	.019–.038	.068–.170	.120	.038		.88
240	1	.038	.106	.919–.038	.102–.238	.136	.040		.88
Birth	1	.048	.108	.02 –.048	.102–.176	.255	.048		.70

[1] Average of a number of measurements.
‡ Irregularly placed.

SMALL INTESTINE

Vacuoles and Diverticula. In the following section of the present paper the development of the intestinal villi and glands has been given especial attention. The vacuoles of the solid stage of the duodenum and intestinal diverticula are briefly described, particularly, because of a probable relation which they bear to the vacuoles found in the œsophagus and the stomach, and because they are closely associated with the development of villi.

The small intestine in the early stages of development of the human embryo is lined with an epithelium which is similar to that of the œsophagus and the stomach. This epithelium is composed of from two to four layers of cells surrounding the central lumen, which in the earlier stages is pervious throughout. The portion of the small intestine which is to become the duodenum begins to grow more rapidly than the lower portion, and in any single embryo may be seen to have attained a higher degree of development. In an embryo of 7.5 mm. the diameter of the epithelial tube of the duodenum is about twice that of the ileum, and its epithelial wall is much thicker. In the older stages, however, this advance in growth of the duodenum is not retained.

In an embryo of 10 mm. the posterior wall of the duodenum is thicker than the anterior and contains for a short distance both above and below the entrance of the ductus choledochus, numerous small vacuoles. Beyond this opening the lumen of the duodenum becomes extremely small, and only a few vacuoles are present. The remainder of the intestine is smaller than the duodenum at this stage, and is more regular and round. Its epithelium, which is composed of three to four layers of cells and is about half the thickness of the duodenum, does not contain vacuoles.

Tandler ('00) described similar vacuoles in the duodenum of embryos of from 30 to 60 days. He found that their formation in some cases led to a complete occlusion of the duodenal lumen, and believed that failure of the lumen to open again caused the well-known anomaly of duodenal atresia. Minot ('00) described a like condition in the large intestine of the chick. It is evident from his drawings (figs. 3–5) that the lumen of the large intestine of the chick is more completely occluded than that of the human duodenum. Forssner ('07) found similar vacuoles and duodenal occlusions in human embryos of about 20 mm. and also in the duodenum of rat embryos.

At 16 mm. the epithelium throughout the entire extent of the duodenum is filled with vacuoles which are larger and more numerous than in the former stage. Immediately below the openings of the ducts, the vacuoles are most numerous and the

lumen of the duodenum is totally occluded. Below this region the vacuoles are fewer and the lumen from this point on is open.

A few vacuoles are present in the upper part of the jejunum at this stage (16 mm.) but these are not as large or as distinct as those found in the duodenum. The lumen of the jejunum and ileum, unlike that of the duodenum, is pervious throughout its entire extent, although in the ileum it is much smaller. There have developed in the epithelial wall at this stage a number of outgrowths, which have been described as "intestinal diverticula." These rounded, bud-like structures are constricted at their necks, and extend into the surrounding mesenchyma. They are found in different stages of development and are variable in size. The smallest have diameters of .043 mm., the largest of about .072 mm., which is about $\frac{1}{3}$ that of the small intestine itself. The older pockets are well marked and are six in number, the younger ones are not as distinct and a few are doubtful. These were 11 in number, making a total of 17 pockets in the small intestine of this embryo.

Keibel ('05) noted the presence of similar pockets in embryos of man, Tarsius, pig, and deer, and considered it strange that these structures were not mentioned by Voigt ('99) and Berry ('02). Lewis and Thyng ('08) described diverticula which they found along the small intestine in human, pig, and rabbit embryos. In a human embryo of 23 mm. they counted 33 pockets; at 22 mm. (H. E. C. series 851), 48 pockets. Elze ('09) found in various mammalian embryos, rounded and elongated intestinal pockets similar to those described by Lewis and Thyng. He observed that the pockets are always on the wall of the intestine away from the mesentery, and that those pockets which were elongated and extended along the intestine for a short distance, invariably pointed down the intestine (ab-orally). In the human embryos of the Harvard Collection, so far as they have been examined, the diverticula present the same characteristics. Because of differences in their arrangement and position, Elze puts the diverticula of the duodenum in a separate class from those of the jejunum and ileum.

At 19 mm. (series 819) the vacuoles in the duodenum are larger than those at 16 mm. The lumen is continuous throughout and

is larger both in actual measurement and in proportion to the size of the vacuoles. In the lower part of the duodenum, these vacuoles are somewhat different from those found in the upper part and from those found in the œsophagus. Many of them have corresponding outbulgings of the epithelium on the mesenchymal surface. Some of these are constricted at their necks and form small side pockets, but for the most part they do not open into the main lumen of the duodenum. These "duodenal pockets," if such they may be called, are presumably different from those referred to by Lewis and Thyng, which give rise to an occasional accessory pancreas. They differ also from the intestinal pockets in their position on the walls of the duodenum, their constrictions are not always so well marked, and their cavities only occasionally open into the main lumen. However, so far as size and general appearance are concerned, they resemble the pockets of the jejunum and the ileum. Besides distinct pockets, all gradations between the simple vacuole of the interior and constricted side pockets are found in the walls of the duodenum. These pockets of the duodenum, therefore, probably represent transition forms between the vacuoles of the upper part of the digestive tube and the intestinal pockets of the lower part.

In the jejunum and the ileum at 19 mm., there are 41 diverticula. These have the same general character as those of the 16 mm. stage, but some, and especially those in the lower part of the ileum, are much larger. They are not equally distributed throughout the whole gut but are more numerous in the ileum. None are present in the upper part of the jejunum.

In the duodenum of an embryo of 22.8 mm. the vacuoles are few above the opening of the ductus choledochus. Below this level, fig. 16, they are numerous and larger in proportion to the size of the tube than at 19 mm. Moreover, they do not form duodenal pockets as in the 19 mm. stage. They cause the whole lumen to be broken up, and, as shown by modelling, the lumen in this region is occluded. This stage, therefore, as far as the vacuoles are concerned, resembles the condition found at 16 mm. more than that found at 19 mm. In the jejunum and ileum 33 pockets were counted. Again they vary in size, the largest meas-

uring .084 mm. Three of these pockets are seen in fig. 17. The first one (lettered *a*) is constricted from the intestine on all sides. The other two (*b* and *c*) are elongated forms, and are not entirely constricted at their necks, *b* being a surface view, and *c* an end view. The two latter extend along the intestine and their free ends point ab-orally.

At 24 mm. the vacuoles have disappeared from the upper half of the duodenum, but they are still present in the lower half. In the upper half, well formed villi are present; in the lower half, villi are seen which are fused together at their apices, but spaces are seen between adjacent villi. These spaces are the above described vacuoles. It is evident from this that the disappearance of the vacuoles is associated with the formation of villi. The exact part played by the vacuoles in the formation of villi will be dealt with in connection with the latter structures. In the duodenum of an embryo of 30 mm. the vacuoles have entirely disappeared.

Because of the development of villi, the intestinal diverticula are difficult to count at 24 mm. It is due to these beginning villi that the pockets disappear. Villi arise around the mouth of a pocket, and by their subsequent growth and enlargement, the pocket is gradually lost by being absorbed in their walls. Some pockets are more elongated than others, and on either side of these short longitudinal folds are seen. By a growth of these folds, pockets are likewise obliterated. As the villi develop from above downward, the pockets disappear in the same order. Consequently, in embryos of 29 mm., 30 mm., 32 mm., and 42 mm., the number of intestinal diverticula becomes smaller and smaller. Those in the ileum attain the highest development. As they increase in size their outer surfaces become flattened and later may even become concave. At 30 mm., the diameter of one of the largest pockets measures .108 mm; at 37 mm. .176 mm.

The significance and fate of the diverticula have been discussed by Lewis and Thyng. They conclude that some elongated forms, which are usually found in the duodenum, are the source of an occasional accessory pancreas. The more rounded intestinal pockets usually degenerate, but some may remain to form cysts

and nodules. It has been my fortune to find, in the mid region of an embryo of 134 mm., a large persistent intestinal diverticulum, fig. 23. It resembles in shape the pockets found in the lower part of the ileum in the 30 mm. and 37 mm. stages. It is connected to the intestinal epithelium by a constricted neck. At its widest place it measured .50 mm. in transverse diameter, and at its neck .21 mm. Extending outward from its somewhat concave outer wall are a number of intestinal glands which are similar to those found in other parts of the intestine. Extending into its cavity from the internal surface of the outer wall are a few villi, one of which is large and extends through the neck of the pocket into the lumen of the intestine.

In the duodenum at 134 mm., several epithelial cysts were found, which are entirely cut off from the epithelium of the duodenum. They are spherical in shape, and each has several glands extending from its external surface. The flattened cells which line these cysts give evidence of an internal pressure. In what manner these cysts are formed, I will not venture to say. There is, however, the probability of their having developed by the persistence of the early duodenal vacuoles or pockets.

Development of Villi. Koelliker ('61 and '79) states that at the end of the second month and in the third, the beginnings of separate villi appear. Barth ('68) and Brand ('77) confirmed this view. Voigt ('99), working on pig embryos, describes an irregular breaking up of the heretofore smooth inner surface of the gut wall by means of depressions and furrows. These furrows increase in numbers and run together to form a net-work of canals. From the fields thus marked off arise slight elevations, the first traces of the villi. Berry ('00), studying pig and human embryos, writes:

"The summary given by Oppel shows beautifully the comparative anatomy of the villi and their evolution. In vertebrates of low order, the intestine is smooth, no villi being present. Then appear longitudinal folds, and then all gradations between folds and villi, and finally villi. It is interesting to note that in those intestines in which folds of the mucous membrane are present, they are more numerous and prominent in the upper part than in the lower

part of the intestine. As villi are developed they again appear in the upper part of the intestine first, so stages are found with villi in the duodenum, and only folds in the ileum. Furthermore, when villi alone are present, they are more numerous in the duodenum than lower down. The most striking result in the following out the development of the villi in the human intestine is that they repeat all the stages found from the standpoint of comparative anatomy."

He concludes, "The villi appear first as longitudinal folds. These folds grow larger and then break up into villi."

Forssner ('07) in a study of œsophageal and intestinal atresiae, while not mainly concerned with the development of villi, confirms the work of Berry in that the villi of the human small intestine appear first as longitudinal folds.

In the human embryos I have studied, only in the lower part of the small intestine was I able to find distinct longitudinal folds preceding the formation of villi. In the upper two thirds of the small intestine, villi are found developing as knob-like invaginations of the epithelium.

The first evidences of villi found are in an embryo of 19 mm. Immediately below the pyloric end of the stomach, the duodenal epithelium shows, besides its vacuoles, other irregularities. These are thickenings of the epithelium and invaginations of the epithelium into the lumen of the intestine, and represent different stages in the development of villi. The thickenings vary in size and height, some being twice as thick as the epithelium of the intestine in other places. The invaginations are slight rounded elevations of epithelium, and have developed from the thickenings by a pushing in of the mesenchyma, which now forms the cores of these small villi. That these are villi and not folds is evident because they can be followed through only four to six 23 μ sections and in breadth at their bases they measure from .10 to .14 mm. From these measurements the bases of these elevations are seen to be approximately circular in cross section. Villi are of all gradations in height, one of the tallest measuring .09 mm. (The height of a villus has in every case been measured on the mesenchymal surface, *i.e.*, the depth of the hole which is filled with mes-

enchyma.) Although the villi are somewhat more numerous in the duodenum, where the diameter of the epithelial tube is large, they are also found in the greater part of the jejunum. Following down the jejunum, the larger villi become fewer and fewer until a region is reached which shows only thickenings. Below this point the lumen of the intestine is smaller and rounded and remains this way until the large intestine is reached.

At 22.8 mm. the villi are larger and more numerous than at 19 mm. They are, as shown in fig. 15, irregularly shaped processes. Below the openings of the ducts, villi—if it can be assumed that they are present—are not recognizable from an inner view of the gut cavity. This is due to the occlusion of the duodenal lumen and to the occurrence of the large vacuoles. However, the outer surface of the epithelium of this region is marked with pits filled with mesenchyma. These pits are similar to those forming the cores of distinct villi found in the other parts of the intestine. It can be inferred from this, therefore, that the formation of villi is taking place while the vacuoles are present in the epithelium, but the individual villi are not distinguishable during the solid stage of the duodenum.

Just below the vacuolated region of the duodenum, true villi are found at this stage (22.8 mm.). They extend into the first loop of the jejunum, but the villi of the duodenum, having developed first, are the larger. As the jejunum is followed down, the villi become smaller and smaller, until a level is reached in which villi are absent. The villi developing in this lower portion of the small intestine are as young as any in the whole gut. In order to make an accurate study of their development, a wax reconstruction was carefully made of this region. Fig. 18 shows half of the model, *a*, looking at the outer surface of the epithelium; *b*, at its inner surface. An examination of the inner surface shows the presence of a number of rounded, knob-like structures, the beginnings of the villi. These are only seen in the upper part of the model. Thickenings of the epithelium are seen in a region below the villi, but at the extreme end of the tube there are no evidences of villi at all. By comparing the external surface of the epithelium, *a*, with the internal surface, *b*, it is seen that there are depressions on

the outer surface to correspond with the villi. The thickenings of the epithelium may or may not have corresponding slight depressions. This model indicates that each villus originates as a thickening of the epithelium, and that later this thickening is invaginated. Still later the epithelium of the invagination becomes thinner and is reduced to a single layer of cells. Below the region of thickenings, the lumen of the intestine becomes smaller and rounded. In no place could longitudinal folds of the epithelium be found.

The idea that villi formation goes on simultaneously in that portion of the small intestine (duodenum) which is vacuolated and in that portion which is not, is further supported by the condition found in the duodenum at 24 mm. In this embryo, only the lower portion of the duodenum shows vacuoles. In the upper part of that region which was previously occupied by vacuoles, villi are easily recognized. Further down there is a region in which are seen a number of villi which are fused together at their apices, that is, end to end. In some places this fusion has the form of thin membranes extending from villus to villus. The formation of the villi seems to be taking place by a pulling apart of preformed villi, and in this process the vacuoles are lost by becoming a part of the lumen. The process of separation of the villi progresses from above downward.

In the mid region of the small intestine at 24 mm. (fig. 19), the villi are seen as separate knob-like elevations of various shapes and sizes. For the most part they have nearly circular bases, but some are elongated. Their apices are rounded. The average of a number of measurement shows the breadth of a villus to be about .11 mm. The tallest villi found in this region measured .12 mm. The epithelium on the tops of the villi is single layered, although in places it appears pseudo-stratified, and is .020 to .025 mm. in thickness. Between the villi are epithelial thickenings which measure .064 mm. in thickness and are composed of from 3 to 5 layers of cells. An examination of the whole of the model (half of which is seen in fig. 19) shows that the villi are arranged in five longitudinal rows. In the lower part of the intestine the villi are smaller; still lower down only epithelial thickenings occur.

Some of these are more elongated than the thickenings found above and form slight irregular ridges. These ridges have as yet no corresponding depressions of the mesenchyma. Below this, there is a diminution in the size of the intestine, and the ridges—2–4 in number —are irregular and changeable in position. Before the large intestine is reached, the ridges are lost and the lumen of the small intestine assumes a more rounded appearance.

The vacuoles have entirely disappeared from the epithelium of the duodenum at 30 mm. and the lumen is pervious throughout. The villi are even more numerous than at 24 mm. and vary in size, the tallest now measuring about .211 mm. The diameters of their bases vary within the limits of .089 and .146 mm. The villi are very irregular in shape, some flattened at their bases, others rounded. The arrangement of villi in rows is more pronounced than at 24 mm. Additional villi have developed either in between the villi of the rows already present, or by forming additional rows. In some places the rows of villi have the appearance of irregular longitudinal folds, suggesting a fusion of adjacent villi. This apearance is perhaps due to the rapid development of new villi between the older ones.

In the jejunum at this stage a similar arrangement of villi is found, but in its lower part the developing villi are further separated. Following down the ileum, they become smaller and smaller. Then comes a region in which there are only a very few villi and thickenings, these being more scattered than before. Following this, there is a region in which the diameter of the tube diminishes, and in this portion longitudinal folds are present. Further down it widens out again and the folds are lost; this is followed by another diminution. Several of these narrowed places are found in this part of the small intestine, and between them the tube regains its former diameter. In the narrowed portions, rather irregular folds are found, varying from 2 to 4 in number. In the widened portions, very young villi are present, but no folds. Fig. 20 is of a wax reconstruction of a portion of the epithelium at a place where there is a diminution in the diameter of the ileum. In the widened portion, the beginnings of a few villi are seen;

in the narrowed portion, two distinct folds, the tops of which are irregular in height, are present.

In the jejunum at 37 mm., the villi are similar to those at 30 mm., but are now regular in shape. They appear to be fused to a greater extent, and there are more longitudinal rows. In shape the villi vary from small low swellings to rounded or elongated processes, some of which are .214 mm. in height. They are only a little taller in the duodenum than below. Further down, at the entrance of a persistent yolk stalk, the intestine becomes wide. In this widened portion both villi and thickenings are seen. Below this region the diameter of the epithelial tube becomes smaller and irregular longitudinal folds are found. Still further down only low ridges are found which have no corresponding depressions for the mesenchyma. These ridges extend as far as the ileocaecal valve. Practically the same conditions are found in an embryo of 42 mm., there being a few folds in the lower part of the ileum where the lumen is narrow. Where it is wide, villi are found.

In the mid region of the small intestine at 55 mm. (fig. 21), the villi have greatly increased in size and in number. They are, for the most part, rounded conical processes but a few are irregular and blunted. A most striking fact is that the villi are all very near the same size. Since additional villi develop as separate invaginations, one would expect to always find a greater number of small villi than large ones. But the small villi have always been found to be less numerous. It follows, therefore, that their growth must be more rapid than that of the older villi. Additional rows of villi have developed between those already present. At 55 mm. there are about 13 rows in the mid region of the small intestine. The villi of the new rows are as tall as those of the older ones, in fact, the later developed rows cannot be distinguished from the earlier ones. The arrangement of the villi in rows is best seen in a view of the external wall of the epithelium (fig. 22). In the extreme lower end of the ileum at this stage (55 mm.) two or three slight folds are found.

Regarding the further development of villi, there is but little of interest. They gradually increase in number and in size throughout stages from 73 mm. to birth (Tables 3 and 4). In the later

stages they present a variety of different shapes in different parts of the intestine. The villi of the duodenum at 78 mm. are cylindrical, with rounded ends, averaging .23 mm. in height. In the upper part of the jejunum they are blunter and measure .20 mm; in the mid region elongated and slender, .25 mm. in height; while in the lower part of the ileum they are swollen at their apices and measure about .28 mm. In the mid region at 134 mm. (fig. 23), the villi are pointed processes; at 187 mm. and 240 mm., they are more elongated. In the mid region at seven months (premature birth) irregular villi are found, some of which are foliate in shape and grooved on their sides. At birth, the villi of the mid region are likewise very irregular in shape.

In briefly summarizing the development of villi, it may be said that the general tendency throughout the whole of the small intestine is for villi to develop as separate invaginations of the epithelium. Owing, however, to the occurrence of transitory structures (vacuoles, diverticula, and folds) their development is manifested differently in different parts of the intestine. In the duodenum the early growth of the villi is closely associated with and concealed by the vacuolated condition of the epithelium. While the vacuoles are present, the apices of the villi of one wall seem fused with those of the opposite wall. They become distinct when this fusion breaks down. At the same time the vacuoles are lost by entering into the formation of the lumen. Throughout the upper two thirds of the jejuno-ileum, villi are first seen as separate thickenings of the epithelium. These thickenings become invaginated, and the thickened plate of epithelium becomes reduced to a single layer of cells. In the lower third of the small intestine, longitudinal folds precede the formation of villi. However, since the folds are present only in those regions of the intestine which are not expanded, they are probably formed because the outer coats of the intestine have not grown as rapidly as the epithelium. The irregularities seen along the tops of the folds, which are of about the same size as the slight swellings in the expanded portions of the gut, are probably developing villi. The folds may later disappear by a general expansion of the epithelial tube, or by being absorbed by the rapidly growing villi. I find

no evidence that there is a mechanical cutting up of the folds into villi, such as has been described.

The villi of all parts of the intestine later become arranged in more or less regular longitudinal rows. Additional villi develop as separate growths by forming new rows, and also in between the villi of the older rows. In some places their development has gone on so rapidly that the villi of a single row are not entirely separate, and give the appearance of short, irregular longitudinal folds. This appearance is lost in the later stages.

In any single embryo, the larger villi, which are all approximately the same size, are always found to be more numerous than the smaller ones. This indicates that the growth of the younger villi is more rapid than that of the older ones. After they have attained the size of the older ones, however, they develop at a corresponding rate.

The growth of the villi in the duodenum and mid region of the small intestine is shown in Tables 3 and 4. The villi of the duodenum, having developed first, are the taller in the earlier stages. At 55 mm. the villi in the mid region have attained the height of those in the duodenum and from this stage on are larger. The bases of the villi remain approximately constant in size but show a slight initial increase and later a diminution in their diameters. The epithelium on the tops of the villi, which is at first stratified, then simple columnar, and finally simple cuboidal, is much reduced in thickness.

Intestinal Glands. According to Koelliker ('61 and '79) the intestinal glands (Lieberkühn's crypts) arise as hollow outgrowths of the epithelium which push their way into the mesoderm. Barth ('68) did not accept this view of the origin of the glands, but believed that they are formed by the spaces left in between the villi. Brand ('77) believed that an upward growth of the mesenchyma was the active factor in the development of the glands. Minot ('92) and Kollman ('98) agree with Koelliker. Voigt ('99) studied the development of the intestinal glands in the pig embryo, and likewise concluded that they originate as hollow outgrowths of the epithelium which are given off between

the bases of the villi and extend into the mesenchyma. This view was later accepted by Hilton ('02).

So far as can be observed from sections, the results of the present work are in accord with those of Voigt and Hilton. In a wax reconstruction of the intestinal epithelium at 37 mm. (not figured) no evidences could be seen of the glands from an external view of the epithelium. At 55 mm. (fig. 22) the exterior of the epithelium in the mid region of the small intestine shows small knoblike outgrowths. These I have considered to be the very first appearances of the intestinal glands. The cells of these knoblike processes are very granular, distinctly different from the clearer cells on the tops and sides of the villi. In amongst the granular cells can be seen a few scattered goblet or beaker cells. The protoplasm of the goblet cells is clear, their flattened or crescentic nuclei staining deeply. In the duodenum of this stage (55 mm.), the beginning glands are somewhat more pronounced. In embryos of 73 mm., 91 mm., and 120 mm., the glands gradually become more distinct and longer, and the goblet cells which line both the glands and the villi become more numerous. The intestinal glands at 134 mm. (mid region of the small intestine) are shown in fig. 23, and at 240 mm. (duodenum) in fig. 24. Throughout the older stages, the glands increase in length and in number. They are usually simple tubular in form, but some are branched. Measurements of the glands in different stages of development are given in Tables 3 and 4.

Duodenal Glands. Regarding duodenal (Brunner's) glands Koelliker states that their formation begins in the fourth month and that they are originally the Lieberkühn glands. Later they send branches into the submucosa, which, at the end of the sixth month, reach to the muscular layer. Barth found that in the rabbit the duodenal glands sprout off from the Lieberkühn glands as a double outgrowth. Brand ('77) observed Brunner's glands first in a human embryo of three and one half months.

In the duodenum of an embryo of 55 mm. no traces of the duodenal glands could be found, while at 78 mm. (Embryo 142)—three months—a few could be seen in the upper third of the duodenum. They arise from the bottoms of the intestinal glands,

as described by Barth, but may or may not be forked at their origins. They can be distinguished from the intestinal glands by the clearer protoplasm of their cells, by their branching, and by the greater distance that they extend into the mesenchyma. As the muscularis mucosae has not developed, there is as yet no line of division between the mucosa and the submucosa.

In the middle third of the duodenum at 91 mm., no duodenal glands are present. At 120 mm. they are present in the whole of the duodenum. They are more numerous in the upper third than lower down, and branching has gone on to a greater extent. In the upper third of the duodenum at 187 mm. the glands are much larger and the branches of each gland are arranged in groups. At the base, toward the muscularis, there is a slight condensation of the mesenchyma, which appears to have been caused by the downward growth of the gland. Only a few glands are found in the middle third of the duodenum at this stage (187 mm.). Fig. 24, from a wax reconstruction, shows the structure of the duodenal glands at 240 mm. The intestinal glands are the short tubular ones arising from around the bases of the villi; the duodenal glands are larger and more branched. The fewness of the primary sprouts in the area modelled is striking, there being only four for the entire number of branches shown, two of which are quite small. The branches of each gland are arranged in groups, lettered in the figure *A*, *B*, *C*, and *D*.

Folds. As stated before, irregular longitudinal folds of the mucous membrane are found in the ileum in stages ranging from 24 mm. to 55 mm. These folds, however, are only transient, and are in no way related to the folds found in the later stages and in the adult. They have practically disappeared at 55 mm.

Circular folds, which have been studied from longitudinal sections, are found in the small intestines of the older embryos. Whether these folds are formed by the contraction of the musculature, or whether they are the non-effacable plicae circulares (valvulae conniventes) could not be positively determined from cut sections, but from their regularity and constancy, I have considered that they are the latter structures. Their presence was first noted in the mid region of the small intestine at 73 mm., but

here they are rather indefinite and uncertain. In the upper third of the duodenum and in the uppermost part of the jejunum at 78 mm. no evidences of the circular folds could be found, but they are distinct in the mid region of the small intestine. They are quite regularly placed, averaging .78 mm. distant from each other. In height they vary from .17 mm. to .23 mm. At the extreme lower end of the ileum at this stage, no folds are present. None were found in the duodenum at 120 mm. (embryo 203). Slight folds are present in the lower end of the ileum at 134 mm. In a distended portion of the small intestine at 145 mm. (mid region), circular folds were found which measure .06 mm. in height, and at an average distance of 1.87 mm. apart. In the duodenum at 240 mm. two circular folds were found at distances of 4.7 mm. and 7.1 mm. below the pyloric orifice. In the mid region of the small intestine at this stage, folds were found which vary from .25 mm. to .51 mm. in height, and at a distance averaging 1.3 mm. apart.

TABLE 3

DUODENAL EPITHELIUM (UPPER THIRD)

EMBRYO	THICKNESS[1] OF SURFACE EPITHELIUM		DIAMETER OF EPITHELIAL TUBE	VILLI		INTESTINAL GLANDS		DUODENAL GLANDS	
Length in mm.	In Cell Layers	In mm.[3]	In mm.	Height[2] in mm.	Diameter[3] (at bases) in mm.	Length[4] in mm.	Diameter[3] in mm.	Length[5] in mm.	Diameter[3] in mm.
7.5	2–3	.036	.077						
16.	*	*	.176						
19.	2–4	.043	.194	.088	.11				
22.8	1–2	.019	.299	.123	.12				
24	1	.024	.36	.193	.11				
30	?	?	.44	.211	.13				
37	1	.024	.53	.23	.13				
42	1	.021	.65	.25	.13				
55	1	.019	.95	.26	.12	.048	.06		
78	1	.016	1.03	.29	.11	.088	.04	.07	.033
120	1	.015	1.11	.31	.11	.09	.06	.12	.031
187	1	.015	2.7	.47	.09	.14	.05	.22	.031
240	1	.015	3.5	.35	.11	.12	.05	.35	.036

[1] On tops of villi when present.
[2] Tallest.
[3] Average of a number of measurements.
[4] Longest.
[5] Vertical distance below bottoms of intestinal glands.
* Vacuolated stage.

TABLE 4

EPITHELIUM OF SMALL IMTESTINE (MID REGION)

EMBRYO	THICKNESS[1] OF SURFACE EPITHELIUM		DIAMETER OF EPITHELIAL TUBE	VILLI		INTESTINAL GLANDS	
Length In mm.	In Cell Layers	In mm.[3]	In mm.	Height[2] in mm.	Diameter[3] (at bases) in mm.	Length[4] in mm.	Diameter[3] in mm.
7.5	2–3	.024	.052				
16	2	.024	.101				
19	3–4	.052	.141				
22.8	3–5	.052	.158				
24	1	.024	.35	.12	.11		
30	?	?	.28	.18	.11		
37	1	.024	.39	.20	.14		
42	1	.021	.44	.18	.13		
55	1	.020	.75	.26	.13	.036	.06
73	1	.019	1.09	.35	.15	.048	.06
91	1	.024	1.28	.37	.14	.050	.06
120	1	.015	1.40	.56	.11	.060	.06
134	1	.015	2.0	.63	.11	.065	.06
187	1	.015	2.5	.70	.11	.088	.06
240	1	.012	3.2	.70	.07	.10	.04
Birth	1	.012	4.9	.70	.11	.12	.04

[1] On top of villi when present.
[2] Tallest.
[3] Average of a number of measurements.
[4] Longest.

CONCLUSIONS

Œsophagus

1. The œsophagus is at first a simple epithelial tube, the walls of which contain three or four rows of nuclei.

2. In embryos of about 20 mm., vacuoles form in the epithelium but the lumen remains pervious throughout. These vacuoles disappear by breaking into the lumen. This causes the epithelium to become thinner and the lumen to increase in size. The cause of the formation of vacuoles has not yet been determined.

3. Longitudinal folds of the mucosa are constant structures in the œsophagus. In the upper third of the œsophagus the folds are irregular and variable. In the middle and lower thirds there

are four large primary folds. Of these the dorsal and ventral (left and right respectively in the lower part of the œsophagus) develop first; the left and right (ventral and dorsal below) develop soon afterward. Smaller secondary folds, variable in number, appear later at the bases of the primary folds. In the lower part of the œsophagus both primary and secondary folds are twisted through an arc of about 90 degrees in the direction of the hands of a clock. It is probable that this twisting is due to the early rotation of the stomach.

4. Areas of ciliated cells are found in the epithelium of the œsophagus in embryos ranging from 55 mm. to birth. There is both an actual and a relative increase in the amount of surface covered by ciliated cells in embryos up to 187 mm. At birth these areas are relatively smaller. Ciliated cells are absent in the œsophagus of a child of 14 days (seven months premature birth).

5. Cardiac glands are found in both the upper and lower ends of the œsophagus. They begin as small areas of glandular cells, which were first seen in an embryo of 78 mm. Later these areas evaginate, forming small pockets and grooves. Later, a number of tubular glands grow out from these pockets.

6. Œsophageal glands were first observed in an embryo of 240 mm. They grow out from the epithelium through the muscularis mucosae and lie in the submucosa. In contradistinction to the cardiac glands, their glandular epithelium does not develop until after the excretory ducts are formed. At birth the end pieces of the glands have begun to branch.

Stomach

1. A few vacuoles, similar to those of the œsophagus, are found in corresponding stages in the stomach.

2. At 16 mm., the heretofore smooth epithelium shows a number of pit-like depressions, the first appearances of the gastric pits. These rapidly increase in number and many become elongated to form grooves.

3. At first the basal surface of the epithelium shows no irregularities due to the gastric pits. Then appear slight swellings into the mesenchyma. Still later depressions or furrows, alternating with the gastric pits, extend inward into the epithelium from the mesenchymal surface. This brings about a readjustment of the cells, and causes the heretofore 2 to 3 layered epithelium to become single layered.

4. The groove-like pits anastomose with one another to form a network. This network marks out irregular areas of the surface epithelium, which have been described as villi.

5. Growth of the mucous membrane is accompanied by an increase in the number of pits and by an increase in their size. The additional pits develop in between those already formed.

6. Glands, first seen at 120 mm., bud out from the bottoms of the pits. These rapidly increase in number and give off branches. Parietal cells are first distinct at 120 mm.

7. Large longitudinal folds of the mucous membrane occur in the stomach, but are variable in position, number, and size. Their occurrence is probably due to the contraction of the muscularis.

Small intestine

1. Vacuoles occur in the epithelium of the duodenum of embryos ranging from 10 mm. to 24 mm. In embryos of 16 mm. and 22.8 mm. the vacuoles were found to lead to a complete occlusion of the duodenal lumen. In an embryo of 19 mm. some of the vacuoles were found to be arranged so as to form small "duodenal pockets." These resemble the intestinal pockets of the jejunum and the ileum. It is probable that these duodenal pockets are transition forms between the vacuoles of the upper part of the digestive tube and the intestinal pockets of the lower part. In the duodenum at 134 mm. several small epithelial cysts were found. It is possible that these are vacuoles or duodenal pockets which have persisted.

2. In the jejunum and ileum intestinal pockets, similar to those described by Keibel, Lewis and Thyng, and Elze, were found.

Most of these disappear by becoming incorporated in the walls of the developing villi, but some may persist. A large persistent diverticulum was found in an embryo of 134 mm.

3. The vacuoles of the œsophagus, stomach and duodenum, and the pockets of the duodenum, jejunum, and the ileum are related structures. They are all the result of a similar process of growth, which for some indetermined reason, manifests itself differently in different parts of the digestive tube.

5. In the upper part of the duodenum, in the jejunum, and in the upper part of the ileum, the villi originate singly as thickenings of the epithelium which are not preceded by longitudinal folds. These thickened plates of epithelium are invaginated, and later reduced to a single layer of cells.

5. In the remainder of the duodenum (approximately the lower two-thirds) the villi are developing while vacuoles are present, but are not recognizable because of the "solid" condition of the duodenum. The apices of the villi of one wall are adherent to those of the opposite wall. The spaces in between the adjacent fused villi are the vacuoles. By a separation of the apices, the individual villi appear, and the vacuoles become confluent with one another to form a continuous lumen.

6. In the lower part of the ileum low longitudinal folds occur. These folds, varying from two to five in number, are irregular in height and position. Their presence is probably due to the fact that the outer coats of the gut do not grow as rapidly as the epithelium. The irregularities along the tops of the folds are developing villi. Later the folds disappear by an expansion of the epithelial tube or by being absorbed by the rapidly growing villi.

7. As the villi develop they become arranged in longitudinal rows of varying regularity.

8. Additional villi develop as separate growths, either forming new rows between those already present, or arising in the older rows.

9. The growth of the younger villi, up to the size of the largest, is more rapid than that of the older ones.

10. Intestinal glands develop as separate hollow outgrowths of the epithelium. They were first seen in an embryo of 55 mm.

11. Duodenal glands originate as outgrowths from the intestinal glands. They branch repeatedly and the branches of each become arranged in groups. They were first observed in an embryo of 78 mm.

12. Circular folds of the mucous membrane make their appearance in the mid region of the small intestine at 73–78 mm. They become more numerous, more regularly placed, and taller in the older stages.

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PLATE I

EXPLANATION OF FIGURES

1. Wax reconstruction of epithelium of œsophagus, showing vacuoles. *a*, human embryo of 19 mm., series 819. *b*, embryo of 22.8 mm., series 871. × 89.

2 Wax reconstruction of epithelium of œsophagus (region immediately below bifurcation of trachea). Human embryo of 37 mm., series 820. Showing single dorsal fold. ×89.

3. Same, human embryo of 42 mm., series 838. Showing dorsal and ventral folds and beginnings of lateral folds. × 89.

4. Same, human embryo of 55 mm., embryo 249. Showing Greek cross stage. × 89.

5. Same, human embryo of 120 mm., embryo 342. Showing four primary and four secondary folds. Maltese cross stage. × 89.

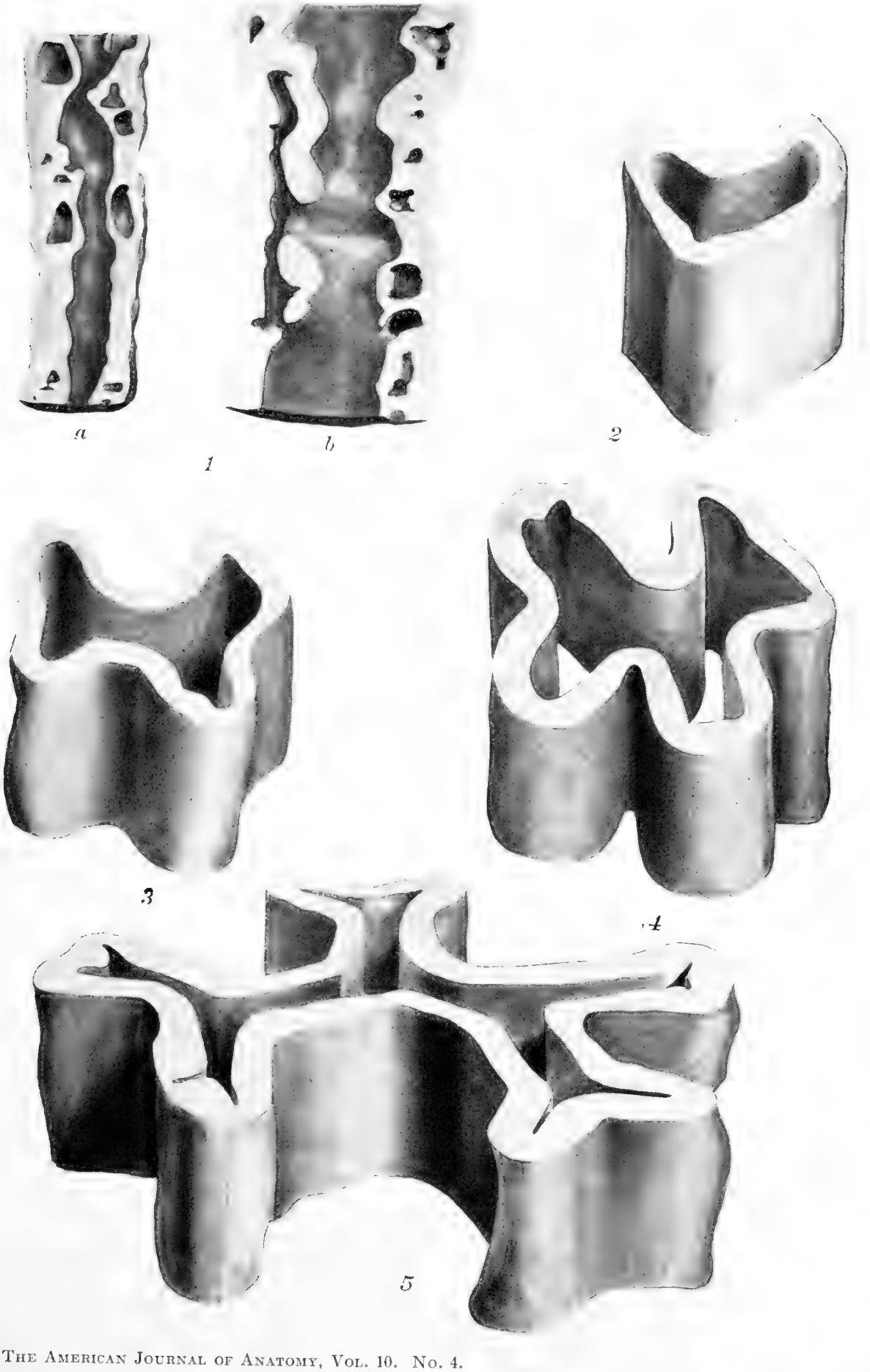
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PLATE II

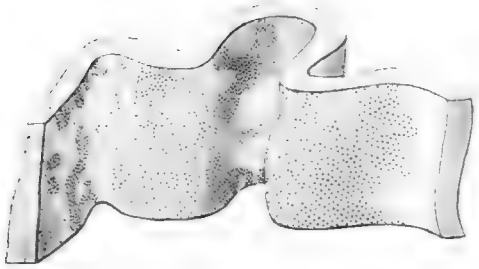
EXPLANATION OF FIGURES

6. Wax reconstruction of epithelium of œsophagus at level of bifurcation of trachea. Human embryo of 55 mm., embryo 249. Stippled areas represent those covered with ciliated cells; non-stippled areas, those covered with squamous cells. × 89.

7. Same, human embryo of 91 mm., embryo 224. × 89.

8. Wax reconstruction of a cardiac gland from lower end of œsophagus. Human embryo of 240 mm., embryo 186. Ruled surfaces represent those covered with glandular cells; non-ruled, those covered with squamous cells. × 89.

9. Wax reconstruction of single fold of epithelium of the œsophagus, viewed from the exterior. Human at birth, embryo 341. Showing œsophageal glands. × 89.



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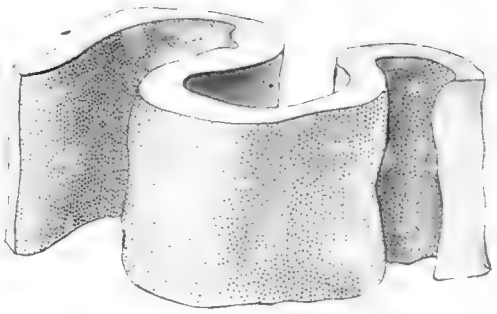

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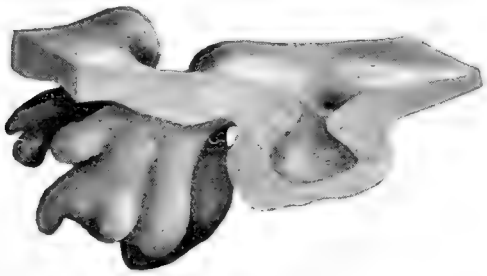

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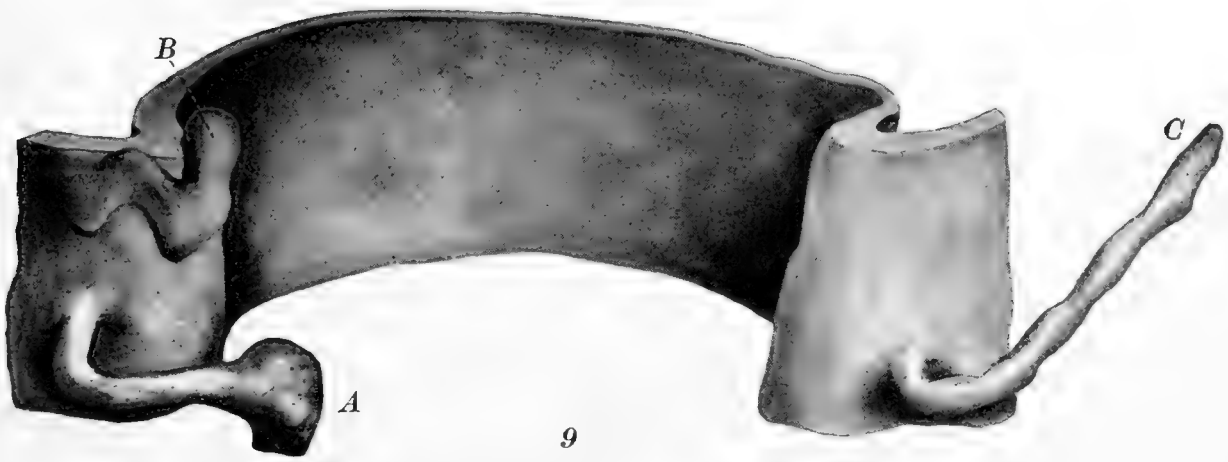

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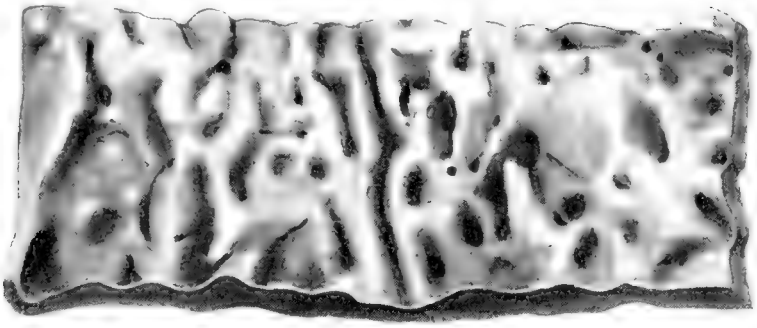
PLATE III

EXPLANATION OF FIGURES

10. Wax reconstruction of epithelium of fundus of stomach. Human embryo of 55 mm., embryo 249. View of internal surface. × 119.

11. Same, human embryo of 120 mm., embryo 342. × 119.

12. View of external surface of same model as shown in fig. 11. Showing gland formation. × 119.



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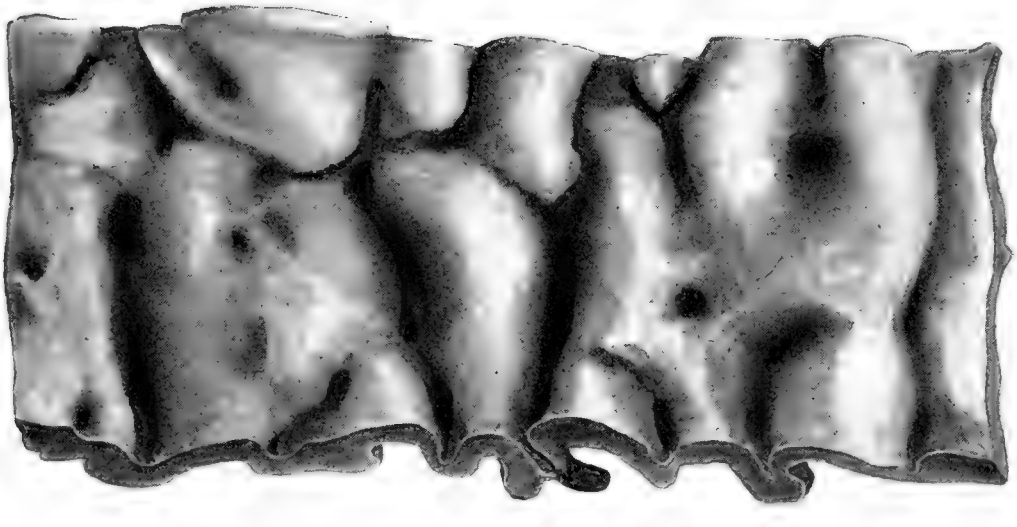

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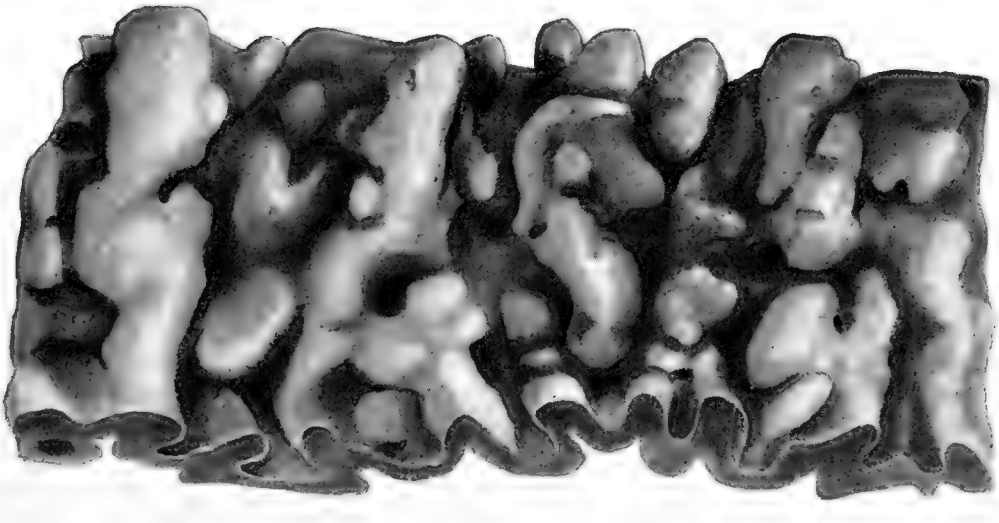

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PLATE IV

EXPLANATION OF FIGURES

13. Wax reconstruction of gastric glands of stomach. Human embryo of 240 mm., embryo 186. × 267.

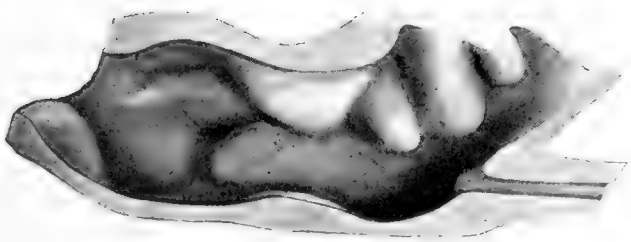
14. Same at birth, embryo 341. × 267.

15. Wax reconstruction of epithelium of upper part of duodenum. Human embryo of 22.8 mm., series 871. Showing beginning villi and duct of the dorsal pancrease. × 89.

16. Wax reconstruction of epithelium of lower part of duodenum. Human embryo of 22.8 mm., series 871. Showing vacuoles and occlusion of lumen. × 89.

13

14



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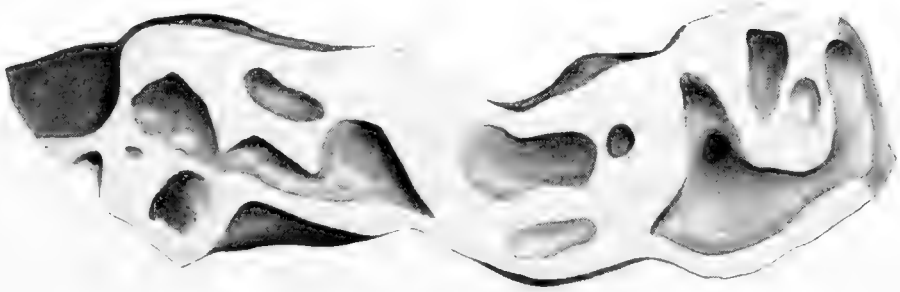


16

PLATE V

EXPLANATION OF FIGURES

17. *a*, *b*, and *c*. Wax reconstructions of intestinal pockets. Human embryo of 22.8 mm., series 871. × 89.

18. Wax reconstruction of epithelium of jejunum (first loop in umbilical cord). Human embryo of 22.8 mm., series 871. Showing developing villi. *a*, external view: *b*, internal view. × 89.

19. Wax reconstruction of epithelium of small intestine (mid-region). Human embryo of 24 mm., series 24. × 89.

20. Wax reconstruction of epithelium of ileum. Human embryo of 30 mm., series 913. Showing low longitudinal folds. × 89.

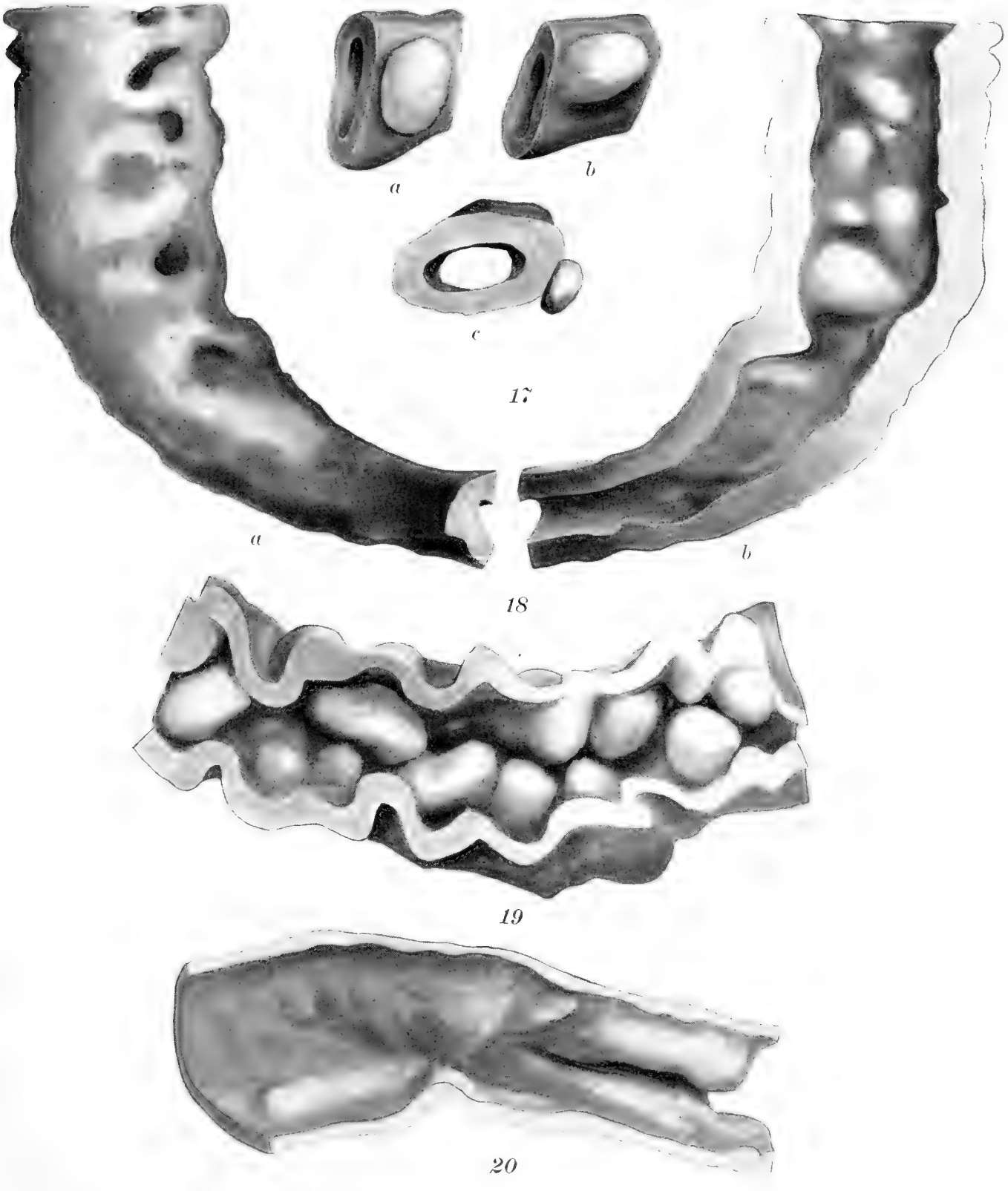

a
b
c
17
a
b
18
19
20

PLATE VI

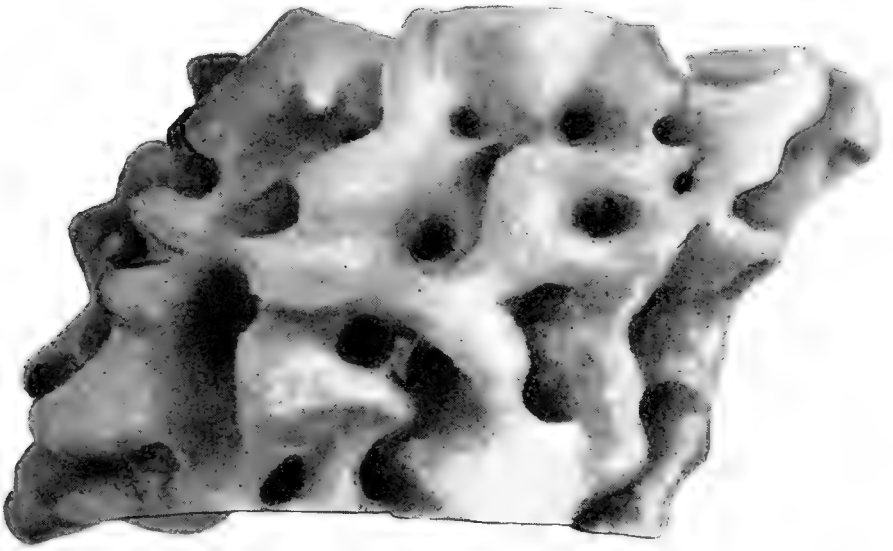
EXPLANATION OF FIGURES

21. Wax reconstruction of epithelium of small intestine (mid-region). Human embryo of 55 mm., embryo 249. Viewed from interior. × 89.

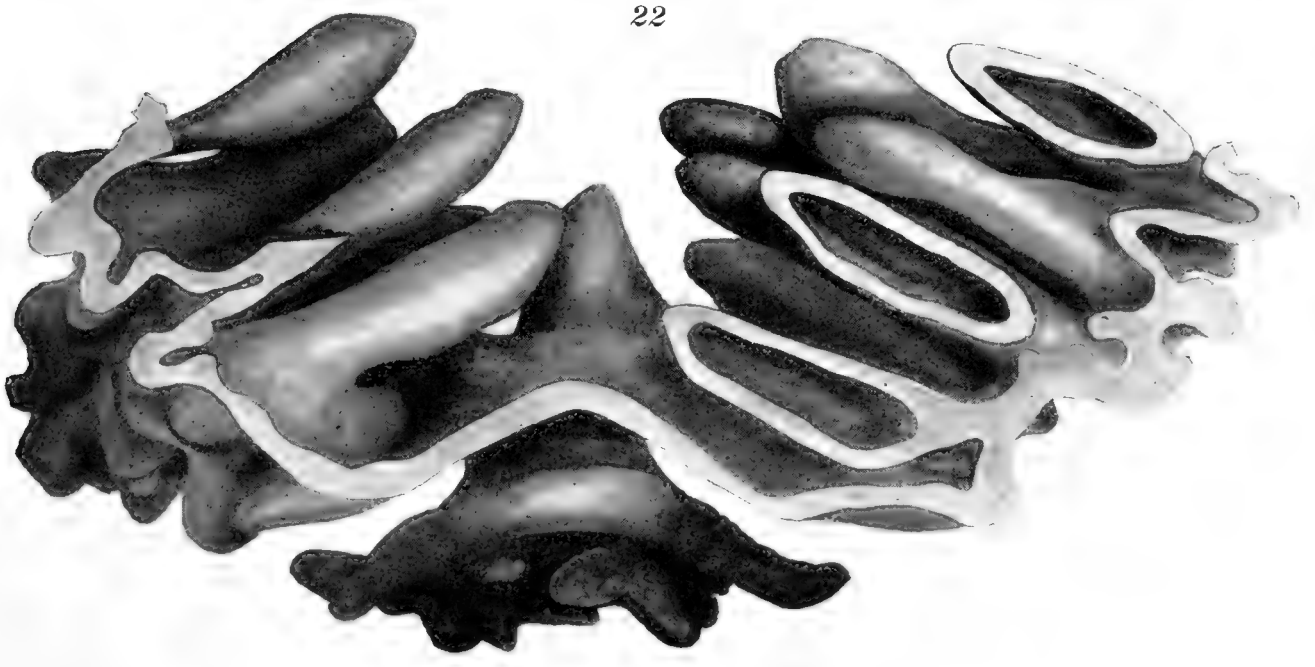
22. Same, viewed from exterior. × 89.

23. Wax reconstruction of small intestine (mid-region). Human embryo of 134 mm., embryo 30. Showing villi, intestinal glands, and persistent intestinal pocket. × 89.

21



22

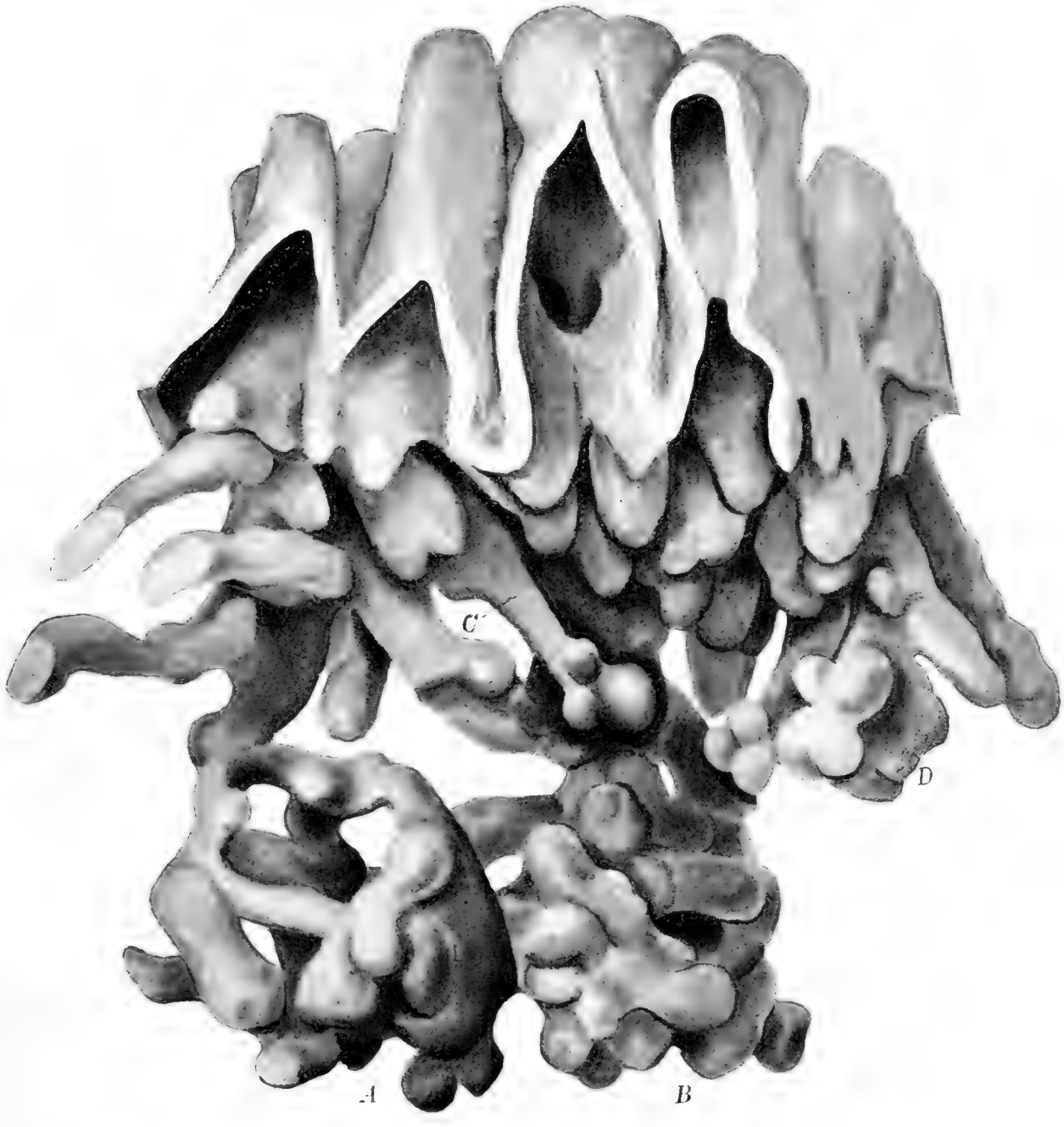


23

PLATE VII

EXPLANATION OF FIGURES

24. Wax reconstruction of epithelium of duodenum (upper third). Human embryo of 240 mm., embryo 186. Showing villi, intestinal and duodenal glands. × 178.



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